

Towards a technology trade war

The European Community set a bad example in its attempt to persuade Japanese electronics manufacturers to export less to Europe. The danger now is that others will follow suit.

WHEN the Black Prince defeated the French at Poitiers in 1356, it must have seemed to many Englishmen that their western bridgehead on the European mainland was secure. But although there were many famous victories such as Agincourt to come, within a century they had been driven back to their islands with their tails between their legs, and Poitiers sank back to being the country town in west central France that it was meant to be. Now, in the past few months, Poitiers has become a famous battleground again, and the scene of another famous victory — that in which the government of France has for the time being won its battle to prevent its own citizens from enjoying such benefits as may be had from buying a video-recorder made in Japan. Prevented as it is by its adherence to the General Agreement on Tariffs and Trade (GATT) from making the import of Japanese video-recorders illegal, the French Government hit on the device of sending all such imports to a warehouse in Poitiers so as to make certain that the shipping documents were in order. Most of the machines diverted to Poitiers are still there, awaiting clearance, but the reputation of the French for being able to play clever tricks on people who happen not to be resident in France has won snickering admiration elsewhere in Western Europe. This, no doubt, is part of the explanation why the British agriculture minister, Mr Peter Walker, felt able two weeks ago to cancel import licences for French-made long-life milk on the grounds that the British Government needed time to tell what steps would have to be taken to ensure that it safe. Technically, the French stratagem at Poitiers, like Mr Walker's little trick, is not an offence against the rules of international trade (in Mr Walker's case, those of the European Community) but a "non-tariff restraint on trade", a way of winning the benefits of protectionism while espousing the cause, and enjoying the benefits, of free trade.

Poitiers

The battle of Poitiers (1982–83) has now been acknowledged to have happened by a kind of treaty, an agreement signed in Tokyo three weeks ago between the Japanese Prime Minister, Mr Nagasone, and Vicomte Etienne Davignon, the European Community's commissioner responsible for industry and, as it happens, research. (M. Davignon last week further confirmed his reputation for being the busiest of all European commissioners by failing to appear as the guest of honour at a luncheon of the British Parliamentary and Scientific Committee; his Irish colleague, Mr Richard Burt, travelled instead from Athens.) The agreement in question is unprecedented in its particularity. It is not about sovereignty but numbers — numbers of video-recorders. Europe has insisted that Japanese companies should not export to the European Community more than 4.55 million video-recorders during 1983, and the Japanese Government has agreed. Moreover, the Japanese Government has agreed that Japanese manufacturers will during the year ahead charge at least as much for their machines as do their less efficient competitors in Western Europe. M. Davignon and his colleagues, meanwhile, have calculated that European manufacturers of video sets will have a captive market for 1.2 million machines, perhaps a fifth of the entire market. The treaty of Poitiers (signed in Tokyo) is everywhere in Western Europe hailed as a triumph for M. Davignon (and for France). It will, however, be more damaging

than advantageous in the long run.

The chief casualties of the Tokyo agreement on video-recorders will not be the Japanese manufacturers of these devices. They may sell fewer machines in Western Europe, but their profit margins will be higher. Potential users of video-recorders will more immediately pay the price of M. Davignon's trip to Tokyo, literally in the higher prices they will be compelled to pay, but they will probably not be aware that the outcome of the negotiations has changed their perception of when they should become purchasers — now, next year or even later? The result of all this diplomacy will be that the market will shrink, that the Western European companies now promised a fifth of it will win less, that the hope of creating in Western Europe an indigenous manufacturing base of a video-recorder industry will be disappointed and that Western Europe will be no better off, either in of its balance of trade with Japan or the proportion of its workforce in employment.

As the Tokyo agreement is set up, Europe will pay much more in yen in 1983 for slightly more video-recorders than European consumers bought in 1982, but will have no permanent advantage as a consequence. M. Davignon should make a point of checking on the figures twelve months or so from now. And exactly the same applies to Mr Peter Walker and his milk. Or to the Californian manufacturers of 64K random access computer memories now complaining that US economic strategy is endangered because others insist on selling the same devices at \$5 each.

Free trade

The truth about free trade is that its benefits are never unalloyed. The economic division of labour helps but also hurts. Historically, it has required that peasant farmers should forgo the luxury of knowing that their own cow will produce the milk, cheese and butter than they need (and the uncertainty that comes from knowing that the cow may die) for the sake of the greater rewards, intellectual as well as economic, of practising their skills. By going to Tokyo to insist that Western Europe has an inalienable right to manufacture a fifth of its own video-recorders, M. Davignon has done little more than to stake a claim that Europe should retain the right to keep its own cow. He is understandably worried about employment, but does he seriously consider that the manufacture of video-recorders (or, for that matter, automobiles) is an essential ingredient of future prosperity? Why not, instead, pour money into the development of less fly-by-night technologies (which include those which in their turn will make the video-recorder obsolete? Because people in M. Davignon's position feel that they must put short-term considerations first.

The European Community is a principal offender against the logic that Adam Smith propounded in another field of high technology — agriculture. Structurally inefficient (too many small farms, too much marginal land in use), European agriculture is nevertheless able to make its way in the world because European taxpayers are compelled to keep uneconomic farmers in business — and are then, to add injury to insult, required to subsidize exports of farm produce as well. In this case, those who suffer most immediately are European taxpayers (who as a result have less to spend on the putative products of a European video-recorder industry) and North American farmers. After three decades, the United States government is now being forced by day-to-day political realities to take up the cudgels in its



own defence. That battle also promises to be bruising and, worse, irrelevant to the future.

If only it were possible, it would therefore be a great advantage if the countries participating in world trade were able jointly, and at this late stage in the slide towards hidden protectionism, to reconsider where they want the world's economy to head. The second instalment of the Brandt Commission's report, published a few weeks ago, pleads again that the poorest nations should be allowed to market simple manufactures (textiles and shoes, for example) more freely than at present; their potential high-tech trading partners resist such claims, however, penalizing their own consumers twice — first in the extra cost of indigenous manufactures, second in the cost of overseas aid (either directly or through commercial banks) which, dictated as it is by the need to spend as little as charity requires, ensures that the recipients are both resentful and unable to be customers for advanced manufactures. Is it entirely out of court that the middle-sized manufacturing states should recognize, as the long recession comes to an end but as unemployment (as it must) deepens, that everybody's interest will be served (and the need for direct foreign aid diminished) if non-GATT trade restrictions are lifted?

The same principle applies in the trading relationships between comparable industrial states, the members of the European Community for example. As things are, all ten members cling to industrial strategies which assume that they will continue for the rest of time to do what they have done profitably in the past, but will also win some clever advantage over their trading partners in novel fields. (Which member of the European Community does not have a national strategy for the exploitation of information technology, *informatique* or some such?) And which fails to operate, in the interests of local employment, a hidden policy on the public purchases of high-technology products which is less than fully economic, which perpetuates for a time technical inconsistencies that will eventually have to be removed by reinvestment and which are also illegal, at least in the sense that they offend against the Treaty of Rome?

The underlying difficulty in calculations of this kind is that governments sense that the consequences of following sensible policies would be politically uncomfortable. A dying industry, or an enterprise that is collapsing, is usually more able to appeal to the hearts and minds of voters than one that does not yet exist. Both in their relationships with each other and with Japan, the nineteenth-century industrial countries of Western Europe are illogically and self-destructively inflexible. (Japan's advantage is its devotion to the concept of the Kleenex industrial strategy — automobiles and ships one decade, electronics including video-recorders the next.) Now that M. Davignon has returned from Japan, and moreover has not had to travel to London, may he not have time to reflect that his Tokyo treaty is a recipe for disaster?

But may not a sensible division of labour hurt too much? That is what the European Community and its member governments are asking. Realism requires that they should be prepared not merely to abandon the belief that past industrial patterns will continue indefinitely but the old-fashioned monetary policies that now beggar their mutual relationships. Mr Peter Walker's policy on imported long-life milk is in this connection no different from but less serious than his government's policy on foreign exchange. Since the discovery of oil in the North Sea, and the oil price rise in 1973, the pound sterling has been strong. The result has been that British manufactures have been partly priced out of export markets but that the British balance of payments has been more or less in balance. The result is that British manufacturing has declined but that at least two successive British governments have been able to boast of sterling's strength. The decline of British manufacturing in the past year has been hastened because the yen has been undervalued, illogically because the United States has chosen to run a huge internal deficit and thus to pay high interest rates on borrowed money. The moral, of course, is that free trade will work only if it is meant to work all over — not merely in manufacturing but (horror) in agriculture and (horror of horrors) in monetary affairs as well. That is the lesson to be learned from this year's pyrrhic victory at Poitiers. □

Reagan's quixotic gesture

Kenneth L. Adelman, nominated director of the US Arms Control Agency, is loyal to a fault.

It is mystifying that President Ronald Reagan should have chosen to make a fight with the US Senate over the issue of whether Mr Kenneth L. Adelman should be next director of the US Arms Control and Disarmament Agency. Adelman was nominated six weeks ago as successor to Mr Eugene Rostow and thus titularly as the inheritor of the common Washington belief that an administration's protestations of commitment to arms control will not be taken seriously unless the director of the Arms Control and Disarmament Agency is at loggerheads with his boss. Mr Adelman's performance in his two appearances so far before the Senate Foreign Relations Committee have been predictably inexpert, even gauche. His confessions that he had not previously wondered what should be done in certain hypothetical circumstances have been, however, more damaging than the possibility that he has lied on oath.

Yet Mr Adelman's nomination stems from the seeming virtues that may yet be his downfall — loyalty, inexperience and gaucheness. Not since Admiral Lewis Straus, once the grand vizier of the US Atomic Energy Commission, was denied the office of Secretary of Commerce in 1959 has a Senate committee so openly defied a president about a nomination. As it happens, Mr Straus was inexpert and gauche in just the ways that have caught out Mr Adelman: he was so loyal to his putative employer that he became arrogant in his scorn of those elected members of the Congress who alone had the power to sanction the payment of his monthly cheque. Mr Adelman, who is younger than was Admiral Straus, should, however in the past week have been more aware of his president's political priorities and more willing to be moved.

The identity of but also the conflict of interest between the president and his nominee are moving. Both men have an obvious wish to ensure that the administration will endure. The president, being older, and better established (or publicly elected) has less need to worry about his future than has Mr Adelman. In the circumstances, Mr Adelman's readiness to fall in with the administration's plans for the agency of which he would be head should be counted as an especially unselfish act; President Reagan might have been better served if Mr Adelman had been able to muster a show of independence.

Ironically, perhaps even pitifully, none of this has much to do with the practical issues that should be worrying the president and his nominee. Before the end of March, the Senate will have a full-scale debate on what should happen about Mr Adelman's nomination and is more likely to make up its mind because of what the telephones from the White House say, or on the basis of what Mr Adelman tells the press, than because of a sober assessment of what the immediate needs are in Geneva, Madrid, Vienna and the other cities in which the United States is engaged in arms control negotiation. The practical issues are, however, clear, and are as follows:

- Renegotiate the verification clauses of the Threshold Test-Ban and Peaceful Nuclear Explosion treaties if that should be feasible, but otherwise ask that the Senate should ratify them before the summer break.
- Keep on insisting on the virtues of the zero-option formula for the control of intermediate nuclear missiles at least until next Sunday evening, when the West German election will be decided, but afterwards be willing to argue for equivalent but unsymmetrical departures from the zero option provided that reduction from planned deployments in Western Europe are matched by the destruction of SS20s on the other side.
- Tell the British and the French that they cannot keep on pretending that their nuclear forces are untouched by the intermediate-range negotiations because they are strategic, but also irrelevant to the strategic negotiations at Geneva because those talks are constitutionally bilateral.
- Do all this before the next important European election has to happen (in Britain?).

Nuclear reactor safety

Europe takes stock of research proposals

Brussels

THE European Commission has been quick to prepare an alternative research programme to replace the ill-fated Super-Sara project on reactor safety based on the Joint Research Centre at Ispra in Italy. But the Commission's proposals are unexciting and uninspiring and must prompt questions about the usefulness of the Ispra establishment, the largest of the Community's four research centres. When the Community's ten science ministers decided to kill off Super-Sara a fortnight ago in Brussels (see *Nature* 17 February, p.554), they said that they would nevertheless maintain the original budget for Ispra. At a press conference to present the new proposals, however, there was no mistaking the anxiety of their chief architects, the Commission's director-general for research and development, Paolo Fasella, and the director-general of the Joint Research Centres, Jean-Albert Dinkespiller.

The new programme will be on the agenda for the next research council on 10 March, but a final decision is not expected until November. A host of consultative bodies, political as well as scientific, will again have to be consulted.

At stake is an annual budget averaging 174 million European Units of Account (£97 million) and a staff of 2,260. The new proposals would concentrate the work of the Joint Research Centres largely on various types of safety research of which between 45 and 50 per cent would be nuclear safety. This share of the budget is not very different from what it would have been if Super-Sara had gone ahead, for under the new proposals more would be spent on nuclear safety research other than Super-Sara. Thus some of the Ispra staff would evaluate the results of reactor safety experiments elsewhere, others would be engaged on putting the Essor reactor into mothballs and still others on theoretical studies of the effects of major external accidents. It is also proposed that work on the safety of fast reactors should be intensified, with the aim of producing at European safety code for nuclear reactors.

One new element in the research programme is the increase in the attention paid to non-nuclear safety research in fields such as acid rain, toxic wastes and industrial risk assessment.

In the long run, the best hope for Ispra is the proposal that it should have a role in fusion research. The ideal, that Ispra might become the site of the next big fusion experiment in Europe, is, however, partly undermined by the prospect that Ispra might become exactly what Dinkespiller

says he wants to avoid — "a juxtaposition of a large number of minor activities".

The reception these proposals get on 10 March will depend on the Italian delegation's position. The council's decision must be unanimous and the Italians are still unwilling to abandon Super-Sara. Whatever the wisdom of a last-ditch struggle at this stage, it has long been evidence to all concerned that the way in which Community research decisions are made needs to be reformed.

Fortunately there is a prospect that this may happen. A paper prepared for the next council meeting proposes that several consultative committees should be scrapped and replaced by one super-committee that would free ministers from the need to discuss the details of the research budget every year. It is also proposed that the Joint Research Centres should be supervised on a day to day basis by a governing board reporting to an administrative council. Sir John (J.B.) Adams, now president of the administrative council, has already been nominated as president of the governing board.

Jasper Becker

Jobs for biochemists

THE Federation of European Biochemical Societies (FEBS) plans to take a leaf out of the book of the large US societies and to organize a job-placement bureau at its annual conference this year in Brussels, from 24 to 29 July. Professor Claude Liébecq, chairman of the organizing committee, says the best way the federation can help young biochemists is to find them jobs.

The venture is a new departure for Europe, but the federation hopes to obtain some valuable assistance from the Federation for American Societies for Experimental Biology, at whose annual conferences the job mart has long been conspicuous.

Liébecq is optimistic about the new scheme. On the telephone this week, he explained that many European companies in need of particular specialist help are unable to recruit scientists from local universities and would be prepared to look elsewhere.

At the same time, it is acknowledged that language problems will be an impediment to recruitment, as will the requirement even in some European Community states that employees should always be nationals. Those seeking to use the new service in July, whether potential employers or employees, should write to the organizers of the 15th FEBS conference, Brussels International Conference Centre, Parc des Expositions, Place de Belgique, B-1020 Brussels, Belgium. □

Scripps clinic

Bloom migrates from Salk

FLOYD BLOOM, one of the most visible of American neuroscientists, is to move closer to Mexico, baby powder and molecular biology. By the end of this year, he will have moved south along the California coast from the Salk Institute to the Scripps Clinic and Research Institute, which has a collaborative agreement with Johnson & Johnson, where he will more easily be able to pursue the molecular approach to the brain that he and Dr Richard Lerner of Scripps have jointly developed over the past three years.

Citing that as one reason for his move, Bloom said it would also be a move back towards the patient. Although he has no plans to reactivate his own medical licence, he is eager to have access to clinical material and the chance to contribute towards the treatment of mental illness and alcoholism. Johnson & Johnson whose net income for the last quarter of 1982 was \$79.4 million — a 10.5 per cent fall on 1981, attributed to the problems of poisoned Tylenol — has recently ploughed at least \$20 million into Scripps in exchange for first rights to any discoveries made there.

Researchers at Scripps have to allow Johnson & Johnson 30 days to peruse papers before they are submitted for publication, for which purpose a number of company employees work within the institute. Johnson & Johnson is also soon to have separate laboratories nearby which will be specifically used to develop Scripps discoveries to the point where their commercial potential can be judged.

One price that Bloom will have to pay for becoming party to the Scripps tie-up with Johnson & Johnson is an exclusive consultancy he has held with Merck, Sharp and Dohme. One that he is unlikely to have to pay, since he expects it to move with him, is the large grant, just renewed for five years, that the National Institute for Alcohol Abuse and Alcoholism gave his laboratory as a designated Alcohol Research Centre.

Bloom hopes to continue that project, still in collaboration with Dr Wylie Vale's group at the Salk Institute. But the main focus of his work at Scripps will be "a comprehensive search strategy to reveal the whole of the gene library that codes for specific neuronal secretory products". It is on that project that Bloom has already collaborated with Richard Lerner, the recent beneficiary of a new department of molecular biology which by this summer will be housed in new buildings paid for by Johnson & Johnson. It was primarily for Lerner's expertise in the development of peptide vaccines that the company invested in the institute; already there is good progress towards a peptide vaccine against hepatitis B and foot and mouth disease.

Peter Newmark

UK energy conservation

Lords say too little too late

A HOUSE of Lords committee is soon to publish a stinging criticism of the British Government's policy on the rational use of energy. The committee's report, now in draft, complains that the government is spending too little on the encouragement of energy conservation, and with too little sense of purpose. But it defends the Government's policies on energy pricing against the recent rumblings of discontent from British industry and the Confederation of British Industry in particular.

The committee is one of several charged with the scrutiny of European Community legislation affecting Britain. To the Community, "rational use of energy" is a technical term embracing energy conservation, pricing policies and the encouragement of conversion from oil to other fuels.

The chief complaint against the British Government is that much of what it claims to be spending in the field — £149 million in 1981–82 — goes on domestic housekeeping (the insulation of public buildings) and that much of what is claimed as research and development is concerned with projects such as new aircraft engines, where energy conservation is a secondary consideration. The committee estimates that support for the rational use of energy in British industry amounts to no more than £10 million a year.

The committee's draft report also complains that the British Government's efforts in the field are poorly led and focused. Ignorance of Government schemes for helping with the cost of conversion to coal, or for supporting demonstration schemes, appears to be widespread, while the committee points out that industries such as brewing (which reduced energy consumption by 11 per cent in a single year and which is aiming at a further reduction of 20 per cent) have made savings largely off their own bat.

The committee asks that the Department of Energy's regional offices should be strengthened, that training courses in topics such as boiler technology (apparently dwindling) should be revived and that the Government should organize itself to help British companies to benefit from a scheme (now being discussed by the European Community) by which rational-use investments would be forgiven 3 per cent of the cost of interest for the first five years. (This scheme is unlikely to be agreed before April.)

The committee's argument about energy pricing is more comforting for the Government. It accepts the view that energy prices charged by nationalized industries should be "realistic", but acknowledges the difficulty of telling what this means when account is taken of the cost of replacing existing fuel sources. It accepts only part of the recent complaint from British industry that energy costs are less elsewhere in the

European Community, saying that the nationalized industries should re-examine their tariffs for large users but that the problems of energy-intensive industries (such as metal processing and paper) may in the long run be insoluble and should in any case be dealt with as part of the Government's energy policy. □

X-ray satellite

Launch to Thor

THE European Space Agency (ESA) has swallowed its political pride and agreed that the X-ray satellite Exosat should be launched from the United States in May on a Thor-Delta rocket and not by means of Ariane. So much is clear from the schedule for the next five Ariane launches drawn up last week. The decision is a success for the astronomers lobbying in the past few months for a Thor-Delta launch.

The lobbying began when it became clear that delays in the Ariane programme could result in the physical deterioration of the satellite's detectors and other problems (such as loss of support staff) (see *Nature* 27 January, p.275). Astronomers had hoped to persuade ESA's council to release Exosat for an earlier Thor launch, but the decision has been left to the last possible moment. The National Aeronautics and Space Administration required notice by the end of February to be able to launch Exosat within its summer window. The Ariane L7 launch, for which Exosat had been planned, will have to follow the next Ariane, L6, and the council has now agreed that there would be too little slack for a summer launch of Exosat to be assured.

The new launch schedule for Ariane has been set after tests of the newly-designed turbopump gearing and lubrication system of the launcher's third stage. A fault in this component was responsible for the failure of the L5 launcher last May. That launch had been intended to place the two satellites in a geostationary transfer orbit and ESA has decided that Ariane L6, to be launched on 3 June, will attempt the same task with the European Communication Satellite ECS1, and Amsat, an amateur radio satellite.

Subsequent Ariane launches have been scheduled for 26 August, 4 November 1983 and January 1984. All three launches (L7 to L9) have been assigned to Intelsat V satellites of the International Telecommunications Satellite Organization. Ariane L10, scheduled for March 1984, will be the first trial for Ariane 3. This, a more powerful version of the rocket, represents an important component in Europe's competition with the United States for the launcher market, and is intended to carry aloft a series of large commercial satellites, some of US origin.

Philip Campbell

Human embryo research

INSERM to ponder ethics

FRANCE is about to plunge itself into the joys — or miseries — of open debate on the ethics of biological and medical research, and particularly the ethics of research with human fetuses. The results, in a country of Roman Catholics and logicians, may be worth watching.

The first step was taken last week with the establishment, by ministerial decree, of France's first "national advisory committee on ethics for the life sciences and health research". This is the brainchild of Philippe Lazar, director of the medical research council (INSERM), who wanted to open up the previous INSERM ethics committee to the public, and to issues beyond INSERM's own particular problems.

Thus the new committee, to be headed by Professor Jean Bernard (a retired haematologist who is an adviser to Lazar), will study the question privately, advise the minister of health, and organize annual public debates on ethical issues in research — the first to take place towards the end of this year. It will also establish an international (and open) documentation and information centre, to compare treatment of ethical issues in different countries and provide a record of particular cases.

The membership of the committee is not yet fixed, but it will comprise four "moralists" (nominated by President Mitterrand) representing different philosophical groups; fourteen representatives of scientific institutions, of whom five will be from INSERM (and two of these from universities), three from the Centre National de la Recherche Scientifique, two more from the universities (independent of research councils), one from the Institut Pasteur and the remainder from other institutions. Another fourteen members will come from affected ministries (such as health, education, and research and industry). Parliament, government and the research councils will each be able to present questions to the committee, which will then be duty-bound to answer; other bodies and members of the public may also pose questions, but the committee will not be obliged to reply.

The question of the moment is the treatment of human embryos and fetuses. Until recently, France had no explicit control of such work (except through local hospital ethical committees, and the special INSERM committee); lately INSERM established guidelines essentially identical to those of the British Medical Research Council (see *Nature* 300, 309; 1982), but these will only be treated as interim.

Meanwhile, the new committee will study the issue, while the ministry of health has published the "draft of a draft" of a law on the medical use of fetuses (avoiding

the problem of *in vitro* fertilization, as the developing baby is technically an embryo to seven or eight weeks after fertilization, and a fetus thereafter).

This embryonic law is primarily concerned with the use of fetal tissue for medical purposes — such as fetal liver for controlling severe immune deficiency in a few-month-old baby, a technique practised (with about 50 per cent success) by Professor Jean-Louis Touraine of the Université Claude-Bernard, Lyon. The draft law demands that a donor fetus must be testified clinically dead by two doctors,

that the mother must agree, that only certain specified and regularly inspected institutions may do the work and that no money should change hands (for fear of sale of fetuses for profit).

The projected law has, however, met with fierce opposition from one quarter — the INSERM research unit on fetal development and the neonate. The director of this unit, Professor Alexandre Minkowski, claims that the death of a fetus is very difficult to determine (cerebral activity being slight or undetectable in many cases).

Robert Walgate

Ordnance Survey

Neither public nor private

THE new British Environment Secretary, Mr Tom King, will be reconsidering the future of the Ordnance Survey following a recent vote in the House of Lords opposing the government's plan for the survey. Their lordships noted "with regret" the government's proposals to set up a Trading Fund to finance the survey along commercial lines. In the course of consultations, users of the Ordnance Survey ranging from the Royal Society to the Ramblers' Association had uniformly expressed their concern that the system would lead to a lowering of standards in the survey's work, which they regard highly.

The Ordnance Survey is a somewhat anomalous body: an independent government department without a minister in charge. In practice, it is looked after by the Department of the Environment (DoE) which drew up the plans. At present the survey is financed on a "vote" system, which means it requests — and gets — funds directly from the Treasury. Under the government's plans, work considered to be in the national interest would be financed on a contract basis by DoE; the rest would have to pay its way. The Ordnance Survey would nevertheless remain a government department. The Royal Society, in its submission to DoE, said "excessive emphasis on the revenue require-

specialist maps. The small-scale maps beloved of countryside users are run on a separate trading account and cover their costs anyway. But large-scale maps, from 2½ to 50 inches to the mile, cannot be sold at a profit, and are currently subsidized by about 70 per cent. The Lords apparently felt, like the users, that turning this subsidy into contract work would leave decisions on future Ordnance Survey projects — such as producing digital maps of Britain — at the mercy of DoE officials. It was also pointed out that the director-general of the Ordnance Survey, as DoE's adviser on cartographic matters, would be in the difficult position of having to advise himself. The matter may now be referred to the new Advisory Board for the Ordnance Survey.

Because of the Ordnance Survey's governmental status, its public relations manager is not allowed to comment on the proposed changes. "We are just getting on with mapping Britain", he said.

Tim Beardley

More bio than tec

THE new journal *Bio/technology*, with a slash or solidus across the "t", made its first appearance this week. The journal is published from New York by Nature Publishing Company Inc., which also publishes *Nature* in the United States. The two journals are, however, editorially independent.

The new journal is probably the most handsome setting yet in which chromatographic gel patterns and nucleotide sequences appear as a matter of routine. The editorial claim of the fastest peer-review system anywhere is well attested by the evidence in the first issue that none of the five research papers to appear was kept waiting more than 17 days for a decision.

The editor of the journal, Mr Christopher Edwards, says that his objective is to concentrate on the technology of biotechnology, but news items in the first issue are mostly concerned with cloning developments and novel monoclonal antibodies. Many readers will, however, be grateful for a review article explaining how

Italy's nuclear power

Action stations

ITALY is to build six 1,000 MW pressurized water reactors, under licence from the American Westinghouse corporation, the government announced last week, putting an end to years of political uncertainty.

Italy has three working power reactors at present, contributing just 1,200 MW between them to the Italian grid. This is only 3 per cent of the total electric power production of the country, which is dominated by oil-burning power stations. The new reactors will replace some of these oil-powered stations, the president of the Italian nuclear agency ENEA, Umberto Colombo, said on Monday.

The breakthrough has come through a new political will to reduce Italy's enormous oil bill, reduced local opposition and a law which gives central government the ultimate right of decision over the placement of nuclear plants.

However, despite regional approval, there is still some stiff local opposition to certain of the six sites, particularly in Lombardy and Puglia.

Meanwhile, some regions have shown themselves willing to accept nuclear power stations. The stage of formal approval has been reached in Piedmont, Lombardy and Puglia. Veneto, Umbria and Basilicata are less well advanced in the approval process.

It will take 14–18 months to select the three sites, and then 7–8 years to construct each reactor. The limited capacity of the Italian nuclear industry means it may be impossible to build all the reactors simultaneously. They may be constructed in two sets of three, but the first should be operating by 1992.

Robert Walgate

bacteria can be used in tertiary oil recovery, while the first issue of the journal includes an analysis of US patents in biotechnology granted during 1982 and abstracts of recent patents.

The chief research article is an account of the nuclear nucleotide sequence of the smaller subunit of the chloroplast enzyme ribulose-1,5-phosphate carboxylase in wheat. Richard Broglie and associates at the Rockefeller University have shown that the smaller protein (eight copies of which appear together with eight copies of the larger protein generated within the chloroplast in the intact enzyme) is coded for by a multigene family in the nucleus.

There is also an account (by a group at the State University of New York at Stony Brook) of how synthetic 29-fluorophytosterols may be effective but safe insecticides because vertebrates lack the metabolic pathway that, in insects, dealkylates the steroid side-chain at C-24, thereby producing toxic fluoroacetate. On balance, *Bio/technology* has less overtly commercial information than might have been expected. □



ment could lead to a loss of completeness and lowering of standards which would be detrimental to scientific research".

The Ordnance Survey has been successfully running joint ventures with private companies for some time, producing

Large Space Telescope

Year's delay in prospect

Washington

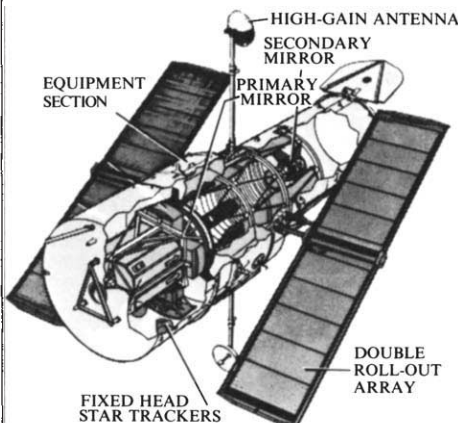
THE Large Space Telescope has encountered technical problems that could delay its launch, scheduled for early 1985, by as much as a year. According to sources close to the project, National Aeronautics and Space Administration (NASA) administrator James Beggs has launched an investigation into the problems that will take two months to complete.

A spokesman for the Marshall Space Flight Center in Huntsville, Alabama, Tim Tyson, said that two kinds of problems have arisen. First, there were delays in installing the mirror into its supporting ring in a strain-free condition. Since the telescope is to be in orbit for many years, it is vital that the mirror is not subjected to possibly deforming stresses.

There are also problems with the precision guidance systems that are to orient the mirror while the telescope is moving in orbit. NASA says that Perkin Elmer Corporation, which is assembling the space telescope, has met the technical requirements but that there are "systematic" delays nonetheless.

The space telescope project has already been in trouble with Congress because of excess costs. If the launch is delayed, further overruns are inevitable, in spite of NASA's promises to the contrary.

In recent weeks, those working on the project are said to have been instructed not to talk publicly about the problems until NASA headquarters completes its investigation. Headquarters is evidently anx-



The configuration of the Large Space Telescope.

ious that rumours of difficulties should not reach Congress before it has made its own investigation.

One casualty of a launch delay could be the plan at the Jet Propulsion Laboratory to use the space telescope to examine Uranus so as to direct the Voyager spacecraft, which approaches the planet in January 1986.

Deborah Shapley

UK science museums

Merger afoot in Kensington

PLANS for a merger between two much-respected London museums look set to go ahead shortly. The British Museum (Natural History) (BM (NH)) and the Geological Museum of the Institute of Geological Sciences (IGS), which occupy adjacent sites in South Kensington, will become one if Sir Keith Joseph, Secretary of State for Education and Science, agrees.

IGS is supported by the National Environment Research Council (NERC), now under considerable financial pressure, and there is much concern that NERC has not been providing adequate support for the museum's educational exhibits given its primary commitment to research. NERC's chairman, Sir Hermann Bondi, denies, however, that the merger is part of a cost-cutting exercise.

The initial impetus for a changed relationship between NERC and the Geological Museum was, according to Sir Hermann, the transfer (still in progress) of the IGS research base from the South Kensington site to Keyworth in Nottinghamshire. It is expected that most of the scientific collections will move to Keyworth, while educational collections — such as its valuable gemstones — will be sold or loaned to BM(NH).

The move is unpopular with IGS staff,

who feel that Keyworth is too remote from London to be a suitable site for what should be a valuable national resource of expertise. But the museum staff who will stay are starting to realize that their educational collections might be better cared for by BM(NH).

Will science education benefit from the merger? The answer is almost certainly yes. BM(NH) already has substantial geological collections (which would complement those of the Geological Museum) and has about 100 geologists on its staff. A plan has already been made to set up a special committee between the IGS and the Natural History Museum to arrange for loans of specimens and to ensure continued access to IGS expertise in such areas as plate tectonics and sedimentology. BM(NH) has also recently appointed as a trustee Sir Frederick Stewart, formerly Regius Professor of Geology at the University of Edinburgh. The main opponent of the change, the Science Museum (also in South Kensington), does not have resident geological expertise. Under the new proposals, BM(NH) — which allocates around 20 per cent of its funds to public display — will be able to set up a truly impressive display covering all of the life sciences.

Tim Beardsley

High-energy physics

Oiling wheels of research

Washington

HIGH-ENERGY physicists, locked in debate on what the next generation of big particle accelerators should look like and who should foot the bill, have not previously suggested that the next machines should also hunt for oil — until last week, that is.

On 22 February, Sheldon ("Shelly") Glashow, the Harvard physicist, presented a plan he has been developing for two years with Robert Wilson, the builder of the Fermilab accelerator, for a 10 terra-electron volt (TeV) proton-antiproton accelerator that will not only put the United States in the forefront of world physics but also shoot a neutrino beam through the Earth to search for oil and heavy metals. They also propose floating their accelerator out to sea to produce a scan of the Earth's interior rather in the way computer-assisted tomography (CAT) produces scans of the human body.

Appropriately, Glashow revealed his oil-hunting accelerator — which others have nicknamed the "Oilatron" — at Texas A & M University before an audience of 300 physicists, petroleum people from Houston and Dallas and interested laymen. Reactions ranged from enthusiasm to disbelief.

Glashow has been wooed by Texas A & M, which frankly seeks to have a Nobel laureate on its faculty. But he has as yet received no formal offer, although he and his wife have been house-hunting and rumours of his possible salary put it at \$250,000 a year. The university is very rich, having access to the income from a permanent fund of some \$1,700 million based on oil revenues (see *Nature* 300, 674; 1982).

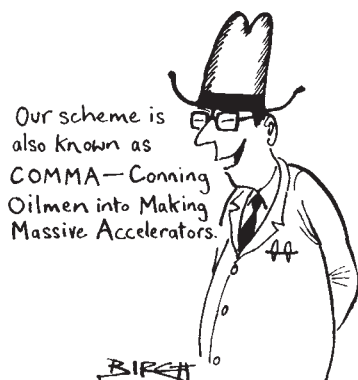
The Oilatron is part of Texas A & M's ambition to become the site of the next large accelerator built in the United States. University officials are now talking with the state of Texas about this machine, which they refer to as the "Texatron". The plan is that both the university and the state could put up part of the cost, thus making the project attractive to the federal government.

Meanwhile, Texas A & M seeks to build a 1 TeV electron-positron colliding linear accelerator along the lines now being studied at the Stanford Linear Accelerator Center, says Peter McIntyre, associate professor of physics at Texas, who went there from Fermilab and Harvard. He hopes the university will make these proposals public in "about a month".

If these ambitious plans materialize (the 10 TeV accelerator would cost at least \$800 million), Texas A & M, which is better known for its marching bands than for physics, will become a world centre. "Texas has big plans for itself. If it is willing to put its money where its mouth is,

it should be taken seriously", says Glashow.

Glashow says he has been working in secret on his project for two years with Robert Wilson and two physicists at the European Centre for Nuclear Physics (CERN), Robert Ch�pak and Alvaro Derujula. They are planning to publish their plan soon. In his lecture, Glashow outlined three kinds of applications. The 10 TeV accelerator would have a circumference of 100 miles. In the search for oil,



code-named GENIUS (for Geological Exploration by Neutrino-Induced Underground Sound), the proton beam would be used to generate neutrinos in a tunnel approximately four miles long directed at the Earth. If, for example, the neutrino beam were pointed down at a 4.5° angle, it would cut a chord through the Earth's subsurface 1,000 km long and 20 km deep at its deepest point. Glashow says that the *in situ* acoustic pulses from a well-directed neutrino beam would be more sensitive indicators of geological structure than ground-based explosions.

The potential of high-precision oil exploration was apparently held out to the petroleum experts who attended last

week's lecture. McIntyre explained to some of them afterwards that "the industry might not have to drill a dry hole again". Heavy hydrocarbons have a much larger expansion coefficient than either water-bearing or dry rock, Glashow explained, so the signal arriving at the surface could indicate clearly whether oil lay in the underground region.

A second application Glashow described is GEMINI, for Geological Exploration with Muons Induced by Neutrino Interactions. This would involve siting ordinary mobile particle detectors where the beam emerges from the Earth. Variations of the accompanying flux of muons are said to be potential indicators of dense ore bodies along the neutrino track.

The third application would in effect be a CAT scan of the Earth's interior — potentially as exciting to geophysicists as its methods would be exotic.

Glashow and Wilson propose putting a 10 TeV accelerator out to sea. The neutrino snout would be pointed down to direct the beam through the centre of the Earth. Neutrino-counters could be located on ships at the antipode where the beam emerged.

Professor Richard Wilson of Harvard University, who is not associated with the proposal, termed it "not completely ridiculous". It's the sort of thing only the oil companies can afford", he said.

Frank Press, the geophysicist who is also president of the National Academy of Sciences, said the proposals would need lots of study and research to determine their practicality — a view Glashow shares. But they might be exciting because "there are two guys, a Nobel prize winner and the world's greatest accelerator builder, going into a new area. That's bound to be interesting."

Deborah Shapley

Polish education

War of attrition

POLISH universities and schools are being used to cripple the young ideologically and morally, General Wojciech Jaruzelski told a party meeting this week. Although, he said, the majority of intellectuals "feel bound to socialism", in some milieus of the intellectual one may still observe "obviously hostile, adventurist and destructive activity", against which "consistent" administrative measures must be maintained.

To some extent, the general's remarks may have been related to the forthcoming trials of the five scholars who acted as "advisers" to Solidarity, three of whom had previously been closely associated with the clandestine "flying university" of the late 1970s. Although the flying university in its old form has not been revived, the underground Solidarity press contains numerous allusions to "alternatives" to the state educational system, in which teachers and lecturers holding official posts may well be participating. From time to time, cases of passive resistance groups organized by academics are reported in the official media, such as the "Fighting Solidarity" group in Wrocław, founded by the mathematician Kornel Morawiecki.

According to underground Solidarity sources, scholars who are suspected of knowing about the composition of these groups and who have refused to pass on this knowledge to the security authorities have been threatened with loss of tenure and loss of bonuses, and have found it impossible to travel to conferences abroad.

For the most part, however, university resistance remains passive. A large number of the rectors elected under the democratic procedures of 1980-81 were replaced during the "verification" process last year — a process which apparently is not yet over, since General Jaruzelski spoke of a "more thorough" phase to take place in the second quarter of this year.

The students, too, seem to have adopted a lower profile — even before Cardinal Glemp's warning to them last week that political activities could jeopardize the forthcoming papal visit. The second anniversary of the founding of the now-illegal Independent Students' Union (NZS) on 17 February apparently passed off peacefully, with only one major protest march, in Krakow, which the police managed to disperse without use of force.

Perhaps the main threat to the universities in the immediate future is not political discontent but the proposed closures next year of intake into 56 higher education courses, and the across-the-board cutback of admissions, which, according to a recent conference of university rectors and party secretaries, is necessary if the standard of education is to be improved, and university courses brought into line with the needs of the economy.

Vera Rich



"Genesis", a structurist portrait by Geoffrey I. Lilley depicting the structure of benzotriazole. Work by Lilley is on view in this year's National Society Exhibition which opened at the Mall Galleries of the Federation of British Artists on 23 February.

East German waste

Water may not be polluted

EAST GERMANY (GDR) "has none of the toxic waste problems reported in other countries", the Minister of Environmental Management and Water Protection, Dr Hans Reichelt, stated recently. He was referring to what the official news agency ADN called "reports by the Western media concerning toxic by-products", presumably a reference to the reports from West Berlin last autumn that the dumping of industrial wastes in East Germany was causing pollution of groundwater, the major part of East Germany's water supply. Heavy metal contamination, it was claimed, was causing special concern.

According to Dr Reichelt, however, dumping takes place only under strict control, and toxic wastes are either partially burned to produce energy or else "stored in such a way as to exclude any danger to the environment".

The West German concern about possible dumping in East Germany has more than a propaganda significance, however. Under the terms of recent agreements between the two Germanies, water is supplied through long-distance pipelines from the Ecker dam which spans the frontier. For his part Dr Reichelt stated specifically that East Germany was "remote from any propaganda demonstration", and was prepared to enter into cooperation with any interested countries.

The East German record over the past few years shows considerable effort. Since the 1960s, agreements with West Germany and with Poland have regulated anti-pollution work in the frontier rivers and, more recently, agreements have been concluded with the Federal Republic on the waters which actually cross the frontier. There are joint projects between the two Germanies on the reduction of pollution in the Steinach and Werra, and the East German government has stated its willingness to engage in consultations on the protection of the Elbe, which, the West Germans complain, is heavily contaminated with cadmium.

Moreover — and this is a far more sensitive issue politically — East Germany recently entered into an agreement with the senate of West Berlin on preventing water pollution. As a signatory to the Helsinki Convention on the Baltic of 1974, East Germany has committed itself to reducing the pollution level of rivers flowing into the Baltic and this is being done, albeit slowly.

The main problem, however, is not reducing the pollution entering the rivers but protecting the groundwaters before they get to the river. Dr Reichelt admitted the urgent need for low-residue technologies, particularly in the chemical, energy, metallurgy, iron ore mining, potash and ceramics industries. **Vera Rich**

Biotechnology

Celltech fights the bottom line

CELLTECH, Britain's own green-field biotechnology corporation, doubled its trading loss in the year to the end of November 1982, but increased its earnings from the sales of products by a factor of five to £384,000, roughly a fifth of the net loss during the year. In the circumstances, it will be widely understood that the company has not made provision in last year's accounts for the ravages that taxation wreaks on profitable companies. Indeed, Celltech's tax losses appear to have increased over the year by £2.1 million, rather more quickly than its accumulated deficit. This seems well in line with the performance of other new biotechnology companies.

The bad news is twofold: Celltech's chairman, Mr H.W. Denham of the British and Commonwealth Shipping Company, one of the original shareholders, resigned at the end of November on the grounds of the time required and the importance of avoiding conflicts between the chairman and major shareholders. But the company has also had to call up a further 29 per cent of the £11.5 million its shareholders had originally promised, so that only a third of

the company's potential capital remains untouched.

The chances that Celltech can get by without asking its shareholders to stump up more than their promised capital therefore depend critically on the company's trading prospects for the present year. The annual report published last week is optimistic about the prospect of rapid growth in the sale of diagnostic aids, among which the reagents for the recognition of the A, B and O blood-groups predominate. The annual report also mentions the manufacture of peptides as a potential growth area, saying that the company will ordinarily offer such developments as licensing packages but citing chymosin (rennin) as one now on offer.

The annual report also gives the lie to rumours that Celltech is a kind of Eldorado for emigrant molecular biologists returning to the United Kingdom. Last year, Celltech's managing director, Mr Gerald Fairtlough, appears to have earned just over £36,000 while Dr Norman Carey, its research director, had his salary increased to more than £30,000 (but less than £35,000). □

US fire research

Dismay at research cutback

Washington

FIRE researchers have reacted with incredulity to a Reagan Administration proposal to eliminate the Center for Fire Research at the National Bureau of Standards (NBS) on the grounds that its work could be carried out by private enterprise or state and local governments.

The centre, with a staff of about 100 and an annual budget of \$5.9 million, is widely respected for its scientific expertise and for its objectivity in an area choked with the competing claims of building-materials manufacturers each arguing the fire safety of its own products. It is also the only major laboratory studying residential fires.

The Administration's proposal to cut off funding for the centre in the 1984 budget has left the fire community more amazed than angry. And at a Senate hearing last week, it became clear just how little thought the Administration had given to the decision. The director of NBS, Ernest Ambler, while being careful not to question the party line, made it clear that no study had been made of the private sector's interest or willingness to take up the research now done at NBS. "I'd hope that they might take up some of it", he said. "You'd hope they might take up some of it?" Senator Slade Gorton (Republican, Washington) asked in amazement.

In fact, according to several key figures in the fire research community, the prospects are exceedingly dim that even part of

the centre's work would find its way into the private sector. The kind of work done at the centre — including the development of standard testing methods for materials and smoke detectors, mathematical modelling of fires, fundamental studies of combustion and flame spread in buildings and studies of smoke toxicity — does not match the capabilities or the interests of private groups, they say.

According to Professor Howard Emmons of Harvard University, a long-time fire researcher, industry laboratories are concerned almost exclusively with testing specific products. Factory Mutual, an insurance group founded by industry to provide industrial fire insurance, runs "probably the best industry fire research system in the country", Emmons says; but "they're small compared to the Bureau of Standards" and "they do nothing about life-loss or toxicity" — only loss of property.

A private organization is also unlikely to supply the \$2 million a year that NBS currently provides for the support of basic fire research at universities.

Emmons added that it is simply unrealistic to expect the residential fire insurance companies to take over the support of fire research: "There is no question that nothing does the fire insurance industry as much good as a humdinger of a fire".

Others pointed to the value of the centre's objectivity — something that private enterprise could not replace. On the issue

of smoke toxicity — which has pitted the plastics industry against the steel industry over the use of plastics in electrical conduit and other building applications — the centre is “the only credible source of technical information”, according to the National Fire Protection Association, a non-profit organization that writes widely-used model fire codes.

Even the plastics industry, which had lodged strong objections to the centre's work on smoke toxicity, issued a statement from its trade association, the Society for Plastics Industry, asking for “serious reconsideration” of the decision to eliminate the centre.

Just why the Administration decided to single out fire research is unclear. Dr Fred Clark, former director of the centre and now a private consultant, said he doubted that concern over the projected \$200,000 million federal deficit could really have been the issue. “All the Reagan Administration has to do is find 40,000 more issues like the Center for Fire Research and they can balance the budget”, he said.

NBS is also facing the loss of its Center for Building Technology in the 1984 budget, a 70 per cent cut in its Institute for Computer Science and Technology (which is developing compatibility standards for the computer industry) and substantial cuts in the Standard Reference Materials and Measurements Assurance Programs, with the funding to be made up by increased “user fees”.

Stephen Budiansky

Saleroom news

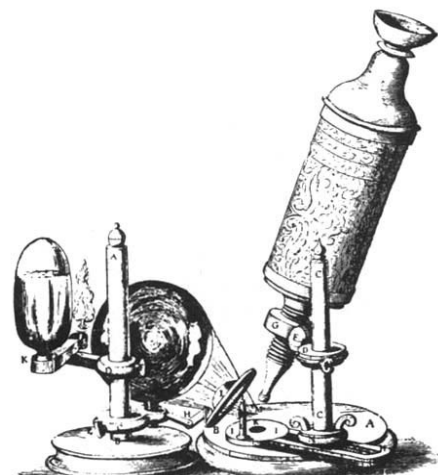
Hooke's classic goes on sale

A FIRST edition of Robert Hooke's *Micrographia*, a work which heralded the birth of scientific microscopy, is to be auctioned this week at Christie's saleroom in London. The auctioneers estimate that the volume will fetch between £3,000 and £4,000, which seems a moderate price for such a rare item.

The full title of Hooke's historic work, first published in 1665, is impressive: *Micrographia, or some physiological descriptions of minute bodies made by magnifying glasses with observations and enquiries thereupon*. It was the first book in English devoted to microscopy and it is enriched by many superb copperplate illustrations in Hooke's own hand. Two drawings in particular — showing a flea and a louse enlarged to about 20 inches in length — created considerable interest when the book was published.

Unfortunately, Hooke's original microscope has not survived but from his extremely clear drawing and description it is seen to have possessed a screw focusing arrangement, and a ball and socket joint providing inclination of the body tube. The stage was an innovation, as was the means of illumination.

Hooke may be remembered in some



Hooke's microscope, as shown in *Micrographia*.

circles for his microscopical observations but today his name is more likely to be associated with his law of elasticity or with his work on the Boylean air-pump. His many other inventions and discoveries seem, for the most part, to be forgotten. Who for instance remembers that it was Hooke who invented the marine barometer and the spirit level, and discovered the phenomenon of diffraction?

He was involved in such diverse matters as the manufacture of artificial fibres, the building of a steam engine and a theory of evolution. And after the Great Fire of London he still found time to lay before the Royal Society a model for the re-building of the City. He was by some accounts first with a Gregorian-type reflecting telescope, a reflecting quadrant and a method of regulating watches by a balance spring.

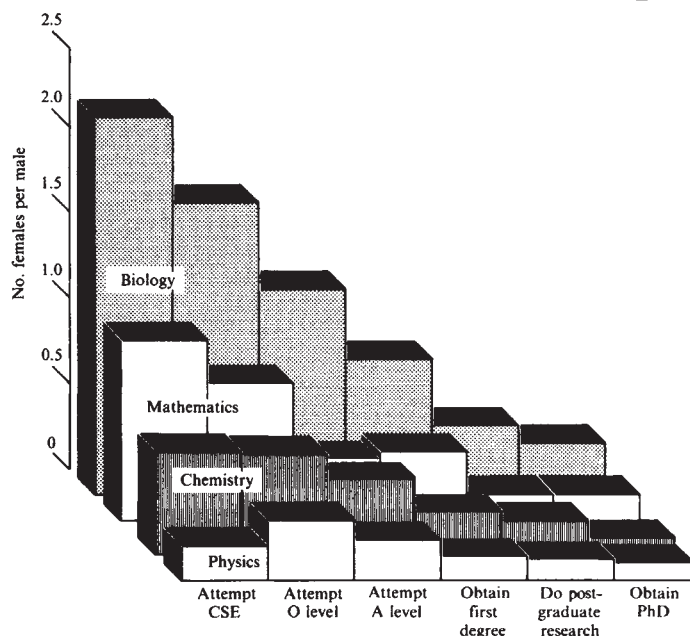
Yet despite his many contributions to science there appears to have been little love lost between him and many of his fellow scientists. It is true that as a Fellow and later as Secretary of the Royal Society he was recognized as a natural philosopher of considerable standing. Yet unsubstantiated charges were made, and repeated *ad nauseam*, claiming that not only did he set too high a value on much of his work, but also that he claimed as his own the inventions of others.

At least some of the bitterness may have stemmed from Hooke's strenuous opposition to Isaac Newton's hypothesis on the different refrangibility of light rays. It was suggested, somewhat uncharitably, that his opposition had been prompted by the rising reputation of this new philosopher working in a field that Hooke looked on as his own. Hooke was subjected to a barrage of abuse, and to hear himself described as “envious, suspicious and of a natural peevishness of character” did not improve his relationship with his fellow scientists.

But whatever may be said of his disposition, he stands out as a leading personality of the seventeenth century and one of the great inventive minds of all time.

Arthur Frank

Women in science in the United Kingdom



LOOKING at the number of females per male studying individual science subjects at various levels of the education system in England and Wales, it emerges that there are more males than females taking almost every pure science subject at all levels. Only elementary biology is studied by more girls than boys, and even this

situation is reversed at degree level. Overall, the same number of girls as boys study science up to the age of 16, after which female representation progressively lessens until, at the postgraduate level, only one in six of those who obtain a PhD is female.

Source: Institute of Physics.

Oxfam's case on drugs

SIR — We are pleased that *Nature* (300, 395; 1982) included a review of our latest publication "Bitter Pills: Medicines and the Third World Poor". But we must take exception to the headline on this piece: "Uncharitable stance from Oxfam". The relief of suffering is central to the concerns of Oxfam and poor people in the Third World are suffering — some dying — for want of essential medicines. Despite the enormous potential of the multinational drug industry to help, some manufacturers are adding to the problems of the world's poor by marketing inessential and wasteful drugs instead of catering for the need for low-priced essential generics.

Your correspondent implies that Oxfam is not helping the poor by publishing this study. Reports from our field staff and project holders in very different countries have made us forcibly aware of the damaging impact on poor people of uncontrolled drug marketing pressures. As this is clearly an issue of considerable public concern we felt compelled to publicize our findings and press for constructive solutions.

Your correspondent places great stock in the fact that "about 50 drug companies are already cooperating with WHO by promising favourable prices" for drugs. In fact all that has been promised is a "preparedness to negotiate on non-commercial prices" for drugs for the public sector. Readers familiar with the vast disparities in drug prices may wonder what concretely is being offered to poor countries. The very real fear is that the *quid pro quo* for low prices for the public sector will be pressure to leave the far more significant private markets largely uncontrolled. In a number of developing countries sales in the private market amount to as much as 90 per cent of total drug distribution. Because of the importance of the private market and the lack of prescription controls, this sector cannot be left uncontrolled if governments are to protect the health of the mass of their people. In 1982 Bangladesh took an important step to give priority to essential drugs by banning those that are inessential. Your correspondent describes this policy as "not . . . likely to encourage further cooperation". The policy may be opposed by industry, but it must be stressed that the Bangladesh government sought to put the health of its people before commercial considerations.

Your correspondent gave the impression that Oxfam's publication is dismissive of the important work in the drugs field undertaken by WHO. Whilst stressing the enormous political constraints under which WHO must operate, we call for more support from governments of the leading drug-producing nations to help implement the resolutions they have already approved — in other words, for real action on the WHO Action Programme on Drugs. Oxfam has put forward positive proposals

to help the Third World poor get the medicines they require. We now await an equally positive response from industry.

DIANNA MELROSE

Oxfam Public Affairs Unit, Oxford, UK

NCI and Frederick

SIR — The *Nature* article entitled "NCI 'mismanagement' causes unrest at Frederick" (20 January, p.185) contains many factual errors in both dollar amounts and mode of operation and also creates the erroneous impression that the multi-contractor environment at the National Cancer Institute-Frederick Cancer Research Center (NCI-FCRC) is proving unsuccessful. We, the undersigned, take great exception to such a judgement, given only several months of experience. We all feel that operational problems are being dealt with in a positive and effective manner. One glaring deficiency in your presentation derives from the fact that four of the contractors, comprising 80 per cent of the total contractor effort, were not contacted. This highly selected sampling invalidates any claims of objective reporting.

The NCI management team here at FCRF, along with Dr Fischinger, associate director, NCI, provides readily available guidance and oversight to ensure that all issues are dealt with in an equitable manner. In fact, it was the NCI staff and not Dr Liverman, as your article mis-states, that established transition and interface meetings to ensure appropriate contractor interaction. These were under way before the contract initiation date to ensure a smooth transition.

We have great confidence in the ability and dedication of the NCI staff to the success of FCRF. We are aware that in any large-scale operation there may be disgruntled employees and occasional thorny issues. These facts of life do not in any way justify any inference of mismanagement. In fact, our signatures, given here freely, reflect the majority view of our staffs that FCRF provides a highly productive environment for cancer research while at the same time being highly responsive to the need for accountability.

RAYMOND V. GILDEN

JAMES L. LIVERMAN

THOMAS W. DAVIS

PAUL A. YOUNG

MICHELE M. SANSBURY

Frederick Operations Program Resources Inc., Litton Bionetics Inc., Harlan Sprague-Dawley Inc., Information Management Services Inc. and Data Management Services Inc.

Deborah Shapley writes — With regard to misinterpretation and errors, the article's text did not mention or imply "mismanagement" at Frederick. The headline, as well as an error in James Liverman's quote, were both copy-editing errors. The article did not say that the new five-contractor arrangement at Frederick

"is proving unsuccessful". Everyone agrees that the proof of success will be long term: whether the science done there continues to improve, whether good scientists can be induced to come, whether good people there now will stay. James L. Liverman, who runs the Litton contract for basic research at Frederick, explained clearly to this reporter that management was doing well considering the unusual and unexpected arrangement that NCI selected for Frederick, with five contractors running five different pieces alongside the laboratories managed directly by NCI. But scientists should judge whether management is effective in this case: if the scientists can work creatively and cooperatively and feel part of the "centre of excellence", that is NCI's stated goal for Frederick. The article said that under this arrangement some scientists are finding it difficult to do their work. This may or may not be a question of "disgruntled employees and occasional thorny issues" found "in any large-scale operation", as the letter says. Four signatories of the letter manage the support services at Frederick and were not contacted on that account.

Morning stars

SIR — Your blanket statement that astrology is sheer superstition¹ is based upon the same bigoted attitudes which were held by the sixteenth century church. I too disagree with the sweeping generalizations produced for the daily media. That is not astrology. A birth chart is based upon a unique permutation of 10 planet positions $\times$ 12 zodiac signs $\times$ 12 "houses" calculated from birth time, birth date and place. Astrology may not be explicable in terms of accepted physical laws, but its credibility lies in the empirical evidence of professionally cast horoscopes valid for the individuals to whom they relate.

Second, the strongest influences in astrology come not from stars but from the planets and their relative angular aspects to the Earth (or Sun). Nelson² correlated specific heliocentric planetary alignments with increased ionospheric disturbance (which in turn disrupted short-wave radio communication). His success rate was 85 per cent.

Vegan diet, talking to plants, and communion with the dead — a widening wedge? Vegans have consistently low plasma cholesterol, high serum folate and a high fibre intake³, factors which can reduce the risk of heart and degenerative diseases. One acre of arable land can produce ten times as much plant as animal protein. This would reduce our dependence on Third World grain which we feed to cattle, and reduce an inefficient food chain.

As for communicating with unseen forces — well, astronomers, chemists and nuclear physicists are doing it all the time. Who can show me an electron?

RICHARD A. BATCHELOR

*Department of Geology,
University of St Andrews, UK*

1. *Nature* 301, 184 (1983).

2. Nelson, J.H. *Elect. Engn.*, 421-424 (1952).

3. Ellis, F.R. & Montegriffo, V.M.E. *Am. J. clin. Nutr.* 23, 249 (1970).

Who knows how massive is the Earth?

The best estimate of the mass of the Earth is uncertain by 6 parts in 10,000 or by 4×10^{18} tonnes. Geophysics is not yet hamstrung, but a better measurement of the gravitational constant would help.

AS the precision with which physical quantities are known has steadily improved in the past few decades, a curious anomaly has become conspicuous. The physical constant that might be thought to be the most interesting of all, the gravitational constant, is much less well known than the others among which it repeatedly crops up.

The difficulty — almost an embarrassment — is nicely illustrated by an attempt, just published by M. A. Khan of the University of Hawaii, to derive from two sets of data recently accepted (no doubt temporarily) in astronomy and geophysics a more accurate value for what is called the dynamical ellipticity of the Earth — the difference between the polar and equatorial moments of inertia of the Earth as a fraction of the former (*Geophys. J. R. astr. Soc.* **72**, 327 and 333; 1983). Khan claims to have inferred from now accepted astronomical data an improved value of the dynamical ellipticity, $(3,273.66 \pm 0.07) \times 10^{-6}$, better by an order of magnitude than previous values. But his attempt to use this value as a springboard for calculating improved values of quantities important in geophysics, such as the mean moment of inertia of the Earth, is for practical purposes defeated by the uncertainty of the Earth's mass or, what amounts to the same thing, the uncertainty in the gravitational constant, still obdurately a few parts in 10^4 .

The ramified origins of these difficulties are interesting and important. Among the fundamental quantities (time, length, mass and something electrical), the measurement of time is now by far the most accurate. For the past decade, indeed, the length of the second has been defined as the reciprocal of a frequency — that of the radiation emitted in the hyperfine microwave transition between ground-state caesium atoms involving antiparallel and parallel orientations of the nucleus and the lone outer electron. The stability of instruments using caesium atomic beams is now 1 part in 10^{14} and standards are reproducible to 1 part in 10^{13} . Building usable secondary standards (clocks) and synchronizing clocks at distant places (now best done by means of satellites) are still substantial difficulties, but the outlook is bright. Better frequency standards than caesium beams are on the cards.

The other primary standards are, by comparison, in a poor state. The pre-laser definition in 1960 of the metre in terms of the optical wavelength of radiation from krypton-86 was, in retrospect, premature;

lasers are at once more stable and more usable standards of length. The general conference of the Bureau International des Poids et Mesures later this year will resolve that dilemma by defining the velocity of light as $299,792,458 \text{ m s}^{-1}$ and letting the metre find its own level thereafter. The mass standard is (relatively) in even worse shape. Any atom (silicon-28 is the favourite) would suffice as a standard, but measuring Avogadro's number is the practical obstacle to constructing usable standards. The electrical standard is in much the same state; the gyromagnetic ratio of the electron is known to one part in 2.5×10^{10} , but converting this knowledge into a usable standard, by means of a Josephson junction or other device, will not be easy.

In the circumstances, it is no surprise that in the interaction between astronomy, geodesy and geophysics, the most precisely known quantities are those ultimately dependent on the measurement of time and on the assumed velocity of light. The System of Astronomical Constants promulgated by the International Astronomical Union in 1976 thus includes values of important parameters of the Earth-Moon-Sun system, such as the various dimensions of the two orbits, or of the Earth's obliquity (inclination of the axis of rotation to the ecliptic), whose uncertainty is typically less than one part in a million, and which is again decreasing rapidly as space-flight improves the measurement of the Solar System.

Yet the masses of the Sun and the planets are unavoidably less accurately known. The dynamical equations of planetary motions involve all interacting masses symmetrically, with the result that only ratios of masses can be determined from observations. In the new IAU data set, the ratio of the masses of the Moon and the Earth (1.230002×10^{-2}) is for example thought to be uncertain by a few parts in a million — and to have been further improved by satellite observations since 1976. Astronomers seem also to be content with the value for the product of the gravitational constant and the Earth's mass given by the data set known as the Geodetic Reference System 1980, given as $3,986,005 \times 10^8 \text{ m}^3 \text{ s}^{-2}$ and thought to be uncertain by 1 part in 10^7 . The snag, of course, is that while the uncertainties in the gravitational constant are only a little less than one part in a thousand, the uncertainty in the mass of the Earth ($5.974 \times 10^{24} \text{ kg}$)

is just as great, to the embarrassment of those who would model its structure.

Khan's objective seems to be to circumvent this difficulty by deriving other dynamical properties of the Earth from astronomical measurements. The dynamical ellipticity is the ratio $(C-A)/C$, where C and A are the polar and equatorial moments of inertia and is a natural (and classical) starting point because it determines the rate of the Earth's precession in its orbit. But, as Khan finds, the uncertainty in the measured precession rate remains about 2 parts in 10^5 , which becomes the residual uncertainty in his value for the dynamical ellipticity. What the Earth modellers need, however, is the mean moment of inertia, and that remains as uncertain as the mass of the Earth, little better than one part in a thousand (Khan says 6 parts in 10^4).

Geophysics will not for the time being be seriously incommode by these persisting uncertainties, given the uncertainty of extrapolating downwards from the surface with only a few benchmarks (such as the depth of the surface of the fluid core) to help *en route*. In principle, and to the extent that seismic velocities at depth are determined in part by the density of the material, evidence of the density profile in the Earth which are independent of gravitational interaction (and thus of the gravitational constant) may eventually be possible — but only when more is known about composition and temperature at depth.

In the circumstances, the best prospects for improving on the accuracy with which the mass of the Earth is known rests almost entirely on better measurements of the gravitational constant. Khan's hope of extracting geophysical information from the precession rate seems doomed to be frustrated by the smallness of the precession (0.14 degrees a century) and by the need to correct Hill's equation relating precession to dynamical ellipticity not merely for well-known complications in the interaction of the Earth with the Sun and Moon but for the fact that the Earth is not, in any case, a rigid body. But the prospect for a substantial improvement in the direct measurement of the gravitational constant is similarly not bright. The gravitational interaction is not for nothing the weakest of the four kinds of forces between particles of matter. And, meanwhile, the mass of the Earth will remain uncertain to the tune of about $4 \times 10^{21} \text{ kg}$.

Cataclysmic variable stars

Why does U Geminorum erupt?

from Marek Abramowicz

SUDDEN appearances of new stars have been reported from ancient times, and John Russell Hind was probably not too surprised when, on 15 December 1855, he found a new star in the constellation of Gemini. Just as expected from the pattern in other such observations, the nova began to fade and disappeared completely two weeks later. Surprisingly, however, in the following March it was seen again at maximum brightness. The unusual reoccurrence caused a great interest among astronomers and the nova, known as U Geminorum, has been carefully observed ever since. Today we know more than fifty stars which show recurrent eruptions similar to those in U Geminorum. They are called dwarf novae and belong to a wider class of cataclysmic variables^{1,2} which includes also classical novae, recurrent novae and nova-like variables (see the table). The more powerful and less common supernovae are not cataclysmic variables.

The cataclysmic variables

Class	log energy of eruption (erg)	Recurrence time
Novae	≥44–45	Only one eruption
Recurrent novae	43–44	10–100 yr
Dwarf novae		
U Geminorum	38–39	15–500 days
Z Camelopardalis	38–39	10–50 days
Nova-like variables	—	No eruptions

Only in the last two decades has it been recognized that U Geminorum is a binary system consisting of a white dwarf (primary) and a late-type star (secondary) transferring mass to the dwarf. The two stars closely orbit each other. The orbital period is very short — only about four hours. Short orbital periods are typical of cataclysmic variables.

The gravitational attractions of the primary and secondary stars are equal at the langrangian point which lies very close to the surface of the secondary. At this point matter is not gravitationally bound and mass transfer from the secondary to the dwarf takes place. The gas streaming from the secondary has, however, too much angular momentum to be accreted directly onto the dwarf and instead forms a rapidly rotating accretion disk around the dwarf in which, due to viscosity, matter loses its angular momentum and slowly spirals inwards. The collision between the stream and the disk produces a bright spot.

The release of gravitational energy by matter accreted onto the dwarf is the main source of energy in U Geminorum.

We see eclipses because the orbital plane of U Geminorum is oriented edge-on with respect to us. Every four hours the secondary star occults both the outer parts of the disk and the spot as it orbits, while the disk occults the spot twice for each orbit. Similar eclipses can be seen in many other cataclysmic variables. This makes it possible to observe separately the properties of the dwarf, disk and spot not only during the long quiescent periods (a few weeks or months) between eruptions but also during the much shorter eruptions (a few days) themselves. From these observations we know that during eruptions it is the disk, and not the dwarf or spot, which brightens. The most detailed empirical model for U Geminorum was elaborated by Smak^{3,4}.

Mass transfer, accretion disks and other concepts tested and confronted with observations in the case of cataclysmic variables are crucial in understanding such other objects as quasars, X-ray binaries, polars and symbiotic stars. It would be hard to overestimate the importance of cataclysmic variables to the rest of astrophysics. Quite ironically, the oldest question, 'why does U Geminorum erupt?', has still not received a definitive answer. Two different points of view have their supporters — some experts believe that the eruptions are due to intrinsic disk instabilities, while others think that rapid changes in the rate of mass loss from the secondary are responsible. The fact that the outermost part of the disk seems to be the key point in this controversy adds much importance to recent work of Hensler⁵, who has developed an accurate numerical method for studying this highly asymmetric and non-stationary region.

The question of the dwarf novae eruptions is surely too exciting to be left for experts alone. Having this in mind, I shall try to present the problem paying equal attention to both points of view. The selection of arguments is based on my own judgements and some of my colleagues will certainly disagree with both my choice and my interpretation of observational data.

Model I: rapid changes in the mass loss rate

Bath⁶ and his co-workers, following earlier suggestions, demonstrated that when the surface of the secondary lies sufficiently close to the langrangian point, bursts in the transfer of mass can occur. In all the dwarf novae, the secondary component has a rather cool envelope (less than 8,000K) which must therefore contain ionization

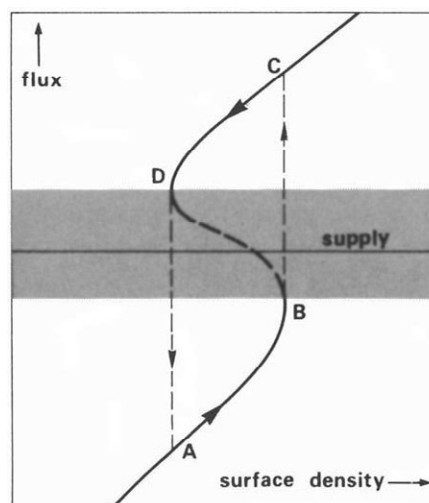
zones. The instability is driven by the release of the recombination energy liberated in the ionization zones as the ionized matter expands, cools and recombines. The location of the surface cycles quasi-periodically between an expanded unstable state and a contracted stable one, and this produces quasi-periodic bursts of mass transfer. Secondary stars in dwarf novae systems have the spectral characteristics expected if this mechanism is to work and the typical time between outbursts given by the model agrees with observations.

Bath and Pringle⁷ computed the response of thin viscous accretion disks for varying mass supply. Assuming various forms of time-dependent behaviour of the supply rate and a rather high viscosity they successfully reconstructed many observed properties of the dwarf novae during eruptions, including the eruption decay time, luminosity and spectral evolution.

Model II: disk instabilities

Osaki and others suggested some time ago that eruptions in dwarf novae are due to disk instabilities but it is only quite recently that the idea has received more elaborate theoretical support. For a given value of the matter flux connected with the inward accretion flow, there is only one corresponding stationary disk structure. In particular, in a given place inside a stationary disk, the value of surface density is always fixed by the value of the flux.

The figure shows how an S-shaped relationship between flux and surface density can give rise to the observed behaviour of the dwarf nova. Points on the heavy line give values of the flux and surface density corresponding to a stationary disk while an unstable branch is shown by the heavy broken line. If the value of matter supply from the secondary, shown by the horizontal line, crosses the surface density versus flux relation in the



Schematic relationship between the flux of accreting matter and the surface density of the accretion disk. If the flux from the secondary star lies within the shaded region, then stable accretion is impossible.

Conservation

Saving the freshwater mussel

from R.J. Roberts

A REPORT by B. Isom and R. Hudson¹ of the Tennessee Valley Authority Water Resources Division that has just appeared in the journal *Nautilus* describes a technique that allowed them to mass culture and transform the larvae of six different species of freshwater mussel. This is the first time that the mass production of mussels from parent stock has been successfully achieved and suggests that the conservation and management of a wide range of the many threatened species of mussel may at last be possible.

Freshwater mussels form a fascinating group of bivalve molluscs with a complex life cycle, usually involving a larvae that is parasitic on the gills of both the female parent and a teleost fish. They are a source of very valuable pearls, and require good water conditions as well as an intermediate host, which is usually highly specific for each mussel.

There has been a drastic decline in their numbers over the past five decades, due to overfishing pressure on stocks themselves, and more seriously, on their intermediate hosts and their freshwater environment. Even in those rivers most renowned for their mussel pearls, such as the River Tay in Scotland, where the environment has been maintained and fishing restricted, mussel numbers are now giving cause for concern.

In many areas of the United States, where half of 1,000 or so known species were once found, most of the surviving species are now listed as threatened or endangered by state or federal agencies.

Artificial propagation of endangered species of mussels has until now been very difficult because of the complexity of their lifecycles. The female mussel produces her eggs and then retains them in the gills where they are fertilized by sperms filtered from the incoming water flow. On the maternal gill the fertilized eggs develop into the characteristic larvae — glochidia — consisting of two tiny shells (250 μ in diameter), a vestigial mantle and a single adductor muscle. The glochidia must then encyst in the fin, or more usually the gill, tissues of a teleost fish and there undergo transformation to the typical adult mollusc. In Scandinavian salmonid hatcheries, it was not unknown for epizootic mortalities to occur following sudden branchial infection of fingerling fish with myriads of glochidia from upstream mussel beds. Recently, however, this has not been a frequent occurrence.

Attempts at conservation and replenishment of endangered stocks have been made, and a method for their *in vitro* maintenance and transformation *in vitro* following excision from infected teleosts

was described by Ellis and Ellis² in 1926. However, the new report from Isom and Hudson is the first that allows the successful mass production and transformation of the glochidia.

Their technique, inspired by the fish tissue culture methods pioneered by Wolf and his co-workers^{3,4}, involves culturing the large numbers of glochidia obtainable from the extirpated gill of gravid females in fish tissue culture media supplemented with fish plasma. Fish cells were found not to be essential for the transformation, although levels of fish plasma up to one-third of the total concentration were essential and could not be substituted by fetal calf serum or other protein source. Bacterial infection of the transforming glochidia was a particularly severe constraint in the early stages of development, but by incorporation of normal levels of antibiotics and mycostatics, up to 80 per cent transformation to viable adult mussels was achieved.

These successes raise hopes that conservation and management of unionid mussels is possible and should stimulate a number of conservation projects and even allow re-colonization of newly rehabilitated rivers where mussels have long been extinct. □

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region of instability, then stationary accretion is impossible. Consider point A. The flux is smaller there than the supply rate, causing local oversupply and an increase of surface density. The disk must evolve in the direction indicated by the arrow up to point B from which further evolution is only possible after a jump to C. Here, however, the flux is higher than the supply. The surface density now decreases and the disk evolves down to point D, where another jump, to A, must take place. This closes the cycle. Between A and B the mass flux is low and this corresponds to the long quiescent periods with low luminosity. Between points C and D the flux is high, corresponding to high luminosity during the short time of an eruption.

Two elements are necessary for this mechanism to work: the curve representing the relation between flux and surface density must have the characteristic S shape⁸ and the value of the matter supply from the secondary must correspond to the unstable part of this curve. Recently Meyer and Meyer-Hofmeister^{9,10}, as well as others¹¹⁻¹³, demonstrated that the first of these conditions is fulfilled in the cool, partially ionized, convective outermost regions of accretion disks in dwarf novae.

This is due to the strong non-monotonic dependence of opacity on temperature which makes the solution for a stationary disk non-unique. The second condition is the condition for dwarf novae: higher supply rates, above the dark instability strip in the figure, correspond to nova-like variables which do not experience eruptions. The intermediate rate, on the boundary of the strip, corresponds to the intermediate case of Z Camelopardalis variables which, in addition to showing the regular quiescence-eruption cycle similar to U Geminorum, occasionally stand still, that is, they remain stationary with luminosity in between that of quiescence and outburst.

Models I and II both suffer from our incomplete knowledge of the theory of time-dependent convection and the mechanism of viscosity. In addition, they assume rather smooth and symmetric matter distribution while real disks are non-symmetric, noisy and lumpy. Attempts to compare theoretical results with very detailed observations of individual events are, thus, in my opinion, premature and the best we can hope for is that our present theories will explain the general properties of both the quiescent periods and the eruptions.

Arguments supporting model I

Bailey found that the decay time of eruptions for dwarf novae lengthens with the binary period. This fits well with the theoretical models of Bath and Pringle because in dwarf novae with longer periods, disks are larger so that more time is needed for a burst of matter connected with a sudden increase in the supply rate to be diffused through the disk down to the dwarf¹⁴. The evolution of luminosity observed during eruptions also closely resembles that computed from the models. To a lesser extent the same is true for the evolution of some spectral characteristics of the light emitted during the eruptions.

On the other hand, existing theoretical models, based on intrinsic disk instabilities, are still not sufficiently developed to allow very detailed predictions of the behaviour of luminosity or spectral characteristics during the eruption. In particular, it is argued⁸ that it would be hard to explain why, in some cases, the luminosity does not change much during the maximal phase of eruption.

Arguments supporting model II

The optical luminosity of the spot is much higher than that of the disk during

quiescence. This suggests that during quiescence, matter is supplied to the outer parts of the disk but only a very little, or no, matter flows through the disk. By studying the changes in luminosities of the dwarf, disk and spot it is possible to estimate that the amount of matter accreted onto the spot during quiescence equals the amount accreted onto the dwarf during eruption¹⁵. This suggests that matter is gradually accumulated in the outer parts during quiescent periods and accreted onto the dwarf during eruptions^{3,4,15-17}. Such a picture, supported by a theoretical model developed by Paczyński, is consistent with other observational data. In my view it gives the strongest argument for the model based on intrinsic disk instabilities.

If the mass transfer rate sharply increases during eruptions, one may expect a simultaneous increase of the luminosity of the spot. It is hard to explain in model I why such an increase is not observed. (Bath *et al.*¹⁸ argue that this is because the stream initially penetrates into the disk.)

So, why does U Geminorum erupt? Most astronomers working in the field favour the model based on intrinsic disk instabilities and I agree with them. Both models, however, have some weak points and they do not explain all the available data. For this reason it would be best, perhaps, not to try to give a categorical answer at this stage. It is likely that the controversy will stay with us for some time to come. Even when, in the future, one model is finally accepted, it should not be taken to mean that theoretical work on the other was done in vain. On the contrary: both models have already contributed greatly to our understanding of cataclysmic variables and, even more importantly, to the development of the theory of accretion disks — one of the most fundamental theories in modern astrophysics. □

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I thank G. Bath and A. Schwarzenberg-Czerny for spending many hours explaining their points of view (very often different from those presented here) and P. Henderson for help in editing the manuscript.

Earth science

Extensive oceanic lava flows?

from Peter J. Smith

BASALT lava flows on land can extend over huge distances. For example, some of the individual flows in the Columbia Plateau lava sequence of the western United States have spread up to 300 km from their source, and others can be traced over tens of kilometres without any noticeable change in thickness. In the oceanic ridge environment, by contrast, such impressive flows have hitherto been thought impossible. It is not that lava flows do not exist in the upper oceanic lithosphere; on the contrary, deep-sea drilling and the study of ophiolites have shown that although oceanic Layer 2A (the igneous layer immediately beneath sedimentary Layer 1) largely comprises pillow lavas, it is interbedded with sediment and sheet lava flows. Such flows are, however, often said to be of limited extent, having resulted from local eruptions whose products fail to travel very far because of rapid cooling by seawater. In the Galapagos rift, for example, the largest sheet flows appear to extend no more than 7 km from the eruptive centre.

Yet the restrictions on the underwater flow of lava may have been overestimated. Some years ago, Harrison and Rooth (in *Volcanoes and Tectonosphere*, Tokai University Press; 1976) showed theoretically that magma extruded at a modest temperature of 1,250°C on land should be able to produce a thin (< 10 m) flow extending 300 km from the source, given a slight slope (10⁻²). They then extended their analysis to underwater flow. As far as the base of a lava flow is concerned, the thermal behaviour is largely independent of environment; heat is lost by conduction to the material below. At the upper surface, on the other hand, a subaerial flow will lose heat largely by radiation whereas an underwater flow will cool largely by convection, although Harrison and Rooth concluded that the rate of heat loss would differ by a factor of less than two. In other words, water may well be less conducive than air to the generation of extensive flows, but not so much less that extensive flows should not form on the ocean floor at all.

With this theoretical result as a starting point, Davis (*Bull. geol. Soc. Am.* **93**, 1023; 1982) has looked at two oceanic regions and finds that extensive underwater flows, though perhaps rare, may indeed exist. The first area is the Juan de Fuca ridge at about 47°N (north-east Pacific). Here the ridge crest morphology is simple and symmetric; there is a central volcanic ridge, probably of pillow lava, resting on a pre-existing valley floor. The floor is for the most part flat and acoustically smooth; and the depth of the

floor is remarkably uniform, varying by less than 30 m in 30 km along the valley axis. This uniformity and smoothness, claims Davis, is precisely what would be expected in a valley floor comprising massive sheet flows stretching over at least the 30 km of the survey region, an interpretation supported by dredge samples and bottom photography.

Unfortunately, an identical effect would probably have been achieved by a series of local lava sources connected hydraulically in such a way that the eruption pressure was everywhere the same; and as there is so far no way of ruling out such a possibility, the existence of extensive flows in that part of the Juan de Fuca ridge must be considered unproven. Some 4° further north, however, in the Dellwood Knolls area of the Juan de Fuca ridge system, the evidence is less equivocal. Here there is believed to be a new (less than a million years old) spreading centre which, unlike most other spreading centres, is covered by thick turbidite sediments from a nearby continental margin (British Columbia). The evidence for extensive lava flows comes from seismic reflection profiles, which indicate the existence on and in the turbidites of numerous lava flows, some of them extending up to 60 km from their source at the Dellwood Knolls. Indeed, some of the flows are so conspicuous in reflection that it would be easy to mistake them for basement, the surface of which is actually well below.

It has to be admitted that the combination of circumstances in the Dellwood Knolls region was unusual. When spreading began, the area was already covered by almost flat-lying turbidites; and rapid sedimentation continued along with the episodic extrusion of lava. The smooth, thermally resistive and mechanically competent sedimentary surface provided ideal conditions for the formation of extensive flows; and the continued sedimentation led to a separation of individual lava flows, rendering them detectable by seismic reflection. Ridge crests are usually free of sediment and, being rugged, leave little scope for unconfined flow. Moreover, even where sediments are present, they do not always have the mechanical strength to support flows (for example, in the Gulf of California). The conditions under which extensive flows can form on the ocean floor are therefore rare but, if Davis's interpretation is correct, they are not non-existent. □

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Cultural evolution

A return to the basics

from Jon Marks, Edward Staski and Michael B. Schiffer

SOME months ago Sir Edmund Leach complained in a book review for *Nature* about the 'rape' of anthropology at the hands of the biological sciences¹. These licentious biologists, however, are not so easily daunted, it seems; and in a recent article in *Science*, Cavalli-Sforza and colleagues² attempt to treat cultural transmission and evolution by strict analogy to biological microevolution. Elsewhere we have been admonished by no less than John Maynard Smith³ that social scientists, who have nothing much to speak of as a theory of cultural evolution, ought not to 'carp and mock' at the suggestions of their biologist colleagues, who, by virtue of their expertise in biological change, may lay claim to the field of social change as well. Presumably, the bulk of anthropological literature must be construed as carping by someone with such an attitude — for very little of it can be judged to support a biologically mechanistic view of cultural processes.

Cavalli-Sforza *et al.* begin by explicitly eschewing a genetical basis for the phenomena they attempt to explore — an admirable and radical admission for many biologists interested in the problem of cultural evolution^{4,5}. Yet such an admission is self-defeating, for as George Gaylord Simpson wrote a quarter-century ago⁶:

"the transfer to the study of cultural evolution within the species *Homo sapiens* of principles dependent on genetic evolution is likely to be metaphorical or analogical, and consequently tricky. Thus natural selection — a genetical process — between cultures or culture elements as such is impossible."

Impossibility, or course, does not daunt geneticists these days the way it used to.

The *Science* paper claims to be analysing cultural values, although the authors use 'cultural' to mean something quite different from any of its meanings in anthropology. The ways in which anthropologists define 'culture' are diverse, but certainly no more so than the myriad ways in which biologists define 'species' or 'gene'. Each definition of culture highlights one or more of the following aspects of human social organization: (1) mediation by symbols; (2) adaptation to social, ecological and intellectual environments; (3) subsistence technology as an independent variable in cultural processes; and (4) culture as a property of societies, not individuals⁷⁻⁹. Cavalli-Sforza *et al.*, however, use the word simply to denote 'the activities, values, and behaviours of an individual that are acquired through instruction or imitation'. Although the authors are correct in pointing out that such learning is 'not exclusively human', they are incorrect in asserting that what they have capri-

ciously defined is 'culture' as the word is regularly used by professional anthropologists.

The bulk of the *Science* article is devoted to the demonstration that (1) in a small sample of college students with remarkable homogeneity of class, race and background, a variety of behaviours generally regarded as matters of taste do indeed vary quite freely; and (2) some religious and political values are systematically passed across a generational line. The authors call these 'cultural' values, but in this case the qualifier is dubious, since the very nature of their atomistic analysis succeeds in leveling out the cultural matrix in which such values are embedded. Their conclusion that son is likely to inherit father's political views and religious inclinations is surely of no novelty and little

value. It does not explain the source of father's views and inclinations, except that, by inference, they arose from grandfather, great-grandfather and ultimately from *Homo erectus*.

Their explanation of human behaviour is a gross reduction to the interaction of pairs of organismal elements, wholly extracted from any determinative matrix of economics, society, beliefs and experiences. Parsimony and rigour are certainly desirable in any scientific enterprise, but we cannot eliminate relevant variables, nor should we sacrifice cogent theory for the sake of quantification and simplification. Moreover, in quantifying and simplifying cultural processes, one runs a risk of trivializing them. For example, anthropologists have long been gaining a comprehension of religion as a complex, multi-dimensional, highly integrated and structured cultural system. Changes in the system necessarily entail social, symbolic, ecological and rational adjustments, since these are all aspects of religious systems¹⁰⁻¹⁴. In contrast, the quantitative methodological breakthrough of Cavalli-Sforza *et al.* determines that if you are a Catholic, Protestant or Jew, then your mother was probably a Catholic, Protestant, or Jew.

Anthropologists are also concerned to delineate the functional nature of political, economic and social aspects of the greater cultural system, and attempt to connect these variables in their dynamic aspects in order to confront cultural evolution. For example, the origins of plant and animal domestication involved fundamental shifts in demography, economic systems, trade networks, settlement patterns, social organizations, religious beliefs and rituals. The trajectory of these changes, furthermore, occurred independently but in the same direction on nearly every continent in both hemispheres¹⁵⁻¹⁹. To an anthropologist, it would hardly be sufficient to explain such changes as mothers teaching daughters a new trick across time and space²⁰.

The *Science* paper goes on to present a discussion of rates of cultural change as a consequence of the ratio of transmitters to receivers: but certainly their analysis confuses cause with effect. Different modes of transmission are a consequence of pervasive organizational features that are rooted in a society's structure. The fluctuating rates of culture change that characterize long-term archaeological sequences (many millennia) are hardly made intelligible by the transmission modes, but rather by examining the interaction between demography, environment, technology and organizational variables²¹⁻²⁵. Unfortunately, Cavalli-Sforza *et al.* do not even acknowledge the need to test their model of culture change against the data of cultures that have changed. Neither in their paper nor in their longer monograph²⁶ do they present any relevant archaeological or ethnographic data. They also do not pro-

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pose any way of measuring accurately such a transmitter/receiver ratio; nor indicate how such a measure might elucidate the kinds of culture change with which anthropologists and historians have to deal.

Further, if we attempt to translate the transmitter/receiver ratio into terms that may be more familiar to social scientists (by equating receivers with initiates and transmitters with elders, without regard to any cultural complexities such as occupational specialization), much of what they seem to be indicating is that a bottom-heavy demographic pyramid leads to rapid cultural change. The view that population pressure as such ordinarily leads to technological innovation, and thus cultural change, has in fact been expounded with considerable skill and erudition, and with appropriate data, by social scientists^{16,19,27-31}.

* Darwin, following Malthus, focused upon the constraining features of the environment in the face of expanding population size as leading to intra-specific competition, and consequently (to Darwin) natural selection. Humans, however, in the face of demographic pressure, can technologically alter the relationship between society and environment. The effect is to mitigate the Malthusian 'struggle for existence', and Darwin's appropriation of it, as a necessary condition for selection.

It is a notable irony that this approach is explicitly anti-Malthusian, and consequently anti-Darwinian!*

Biological parallels to cultural evolution have, in fact, been proposed before, though usually with candid acknowledgement of their limitations³²⁻³⁶. It remains, however, that these biological models are but metaphors, and of no greater applicability *a priori* to cultural processes than are quantum mechanics or the full-court press. Moreover, few of these biological models have been tested, and none appears to be capable of dealing with such fundamental cultural processes as the rise or collapse of civilizations³⁷. Finally, if culture 'evolves' then it is an analogue of the gene pool and the property of a society (as a gene pool is the property of a population); yet, if culture is transmitted and possessed by individuals, it is an analogue of the genotype. Muddling the fundamentals of this analogy effectively precludes its rigorous application. □

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Macroevolution

Large-scale replacements in the history of life

from Michael J. Benton

THE great debate over whether evolution proceeds gradually or in jumps highlights only one area of evolutionary biology where major changes in thinking are taking place. Studies of evolution at the next higher level — the search for underlying pattern in, and explanations for, the replacements of whole families, orders or classes over geological time — have also yielded exciting results. New analyses suggest that the classical view, that some groups die out simply because they become 'out of date' and are replaced by their more efficient competitors over long periods of time, is not an adequate explanation. There is increasing evidence that major physical changes have caused more large-scale evolutionary changes than has competition. At the same time, computer simulations suggest that the apparent pattern in disappearances of whole groups may sometimes be the result of a purely random summing of events at a lower level.

Worldwide replacements of major taxonomic groups have occurred many times during the history of life and palaeontologists have typically sought particular reasons for each extinction event. Such deterministic explanations include both large-scale 'competition' and extinction followed by a new adaptive radiation. Competition at the individual level, or differential survival at the species level, has been the favoured explanation when two taxonomic groups that

apparently occupy the same broad adaptive zone coexist for some time and one increases in abundance at the expense of the other. The alternative view, that certain major changes in the history of life are to be ascribed to changes in the physical environment, is particularly applied to mass extinction events that are followed by adaptive radiations of other groups. These two kinds of explanation rely respectively on biological effects ('competition') and physical effects (causing mass extinction) and of course the two are inseparable in the environment of any species.

A third viewpoint has been advanced that all change at higher taxonomic levels in the history of life is random. Species evolve and become extinct at random, and a set of chance extinctions may lead to the extinction of a whole larger group. The idea has been tested by running computer simulations of evolution, and the random patterns that result are very similar to the patterns of evolution as recorded in the fossil record¹. However, a dissenting viewpoint is that at certain times, for example during adaptive radiations and mass extinctions, there are identifiable causes². For example, during an adaptive radiation the probability of speciation may be enhanced by the acquisition of a new adaptive innovation.

From a theoretical viewpoint, we can suggest three models for large-scale extinction/replacement events: (1) com-

petition/differential survival (biological factors): numerous competitive encounters between individuals or species in which those belonging to one lineage have a constantly higher probability of survival; (2) mass extinction/opportunistic replacement (physical factors): all members of a particular lineage become extinct at the same time and are replaced by another lineage after the extinction; (3) stochastic (random): a mixture of biological and physical factors; at the level of individuals or species, each death/extinction has a particular cause, but the sum is random with respect to the whole lineage.

'Competitive' explanations have traditionally been used by palaeontologists to account for the replacement of brachiopods by bivalves, the replacement of mammal-like reptiles by dinosaurs, the partial replacement of perissodactyls by artiodactyls and the success of North American mammals in South America. In all cases, these explanations have been questioned by a closer study of the fossil record.

Gould and Calloway³ plotted the numbers of genera of brachiopods and bivalves against time and demonstrated that the major extinction events at the end of the Permian (225 Myr) reduced brachiopod numbers more drastically than those of the bivalves. The latter group recovered and radiated rapidly while the brachiopods maintained the same low diversity ever after (Fig. 1). The crucial blow was the extinction event (external physical causes) and not gradual competitive attrition of one group by the other.

The same conclusion seems to hold for the replacement of mammal-like reptiles by thecodontians, and then by their descendants, the dinosaurs, during the Triassic Period (225–190 Myr). A gradual competitive model has also been traditionally assumed, with dinosaur success ascribed to supposedly superior locomotory, feeding or thermoregulatory ability. Plots of numbers of species, or of individual animals, against time show, however, that the carnivorous thecodontians remained unimportant throughout the Triassic and failed to replace their competitors, the cynodont mammal-like reptiles. The dinosaurs radiated rapidly towards the end of the Period, after the extinction of the groups they replaced⁴.

During the Tertiary, the artiodactyls (cattle, antelopes, camels and so on) largely replaced the perissodactyls (horses, rhinos, numerous extinct groups) and this has been explained in terms of the competitive advantage of various characters of their limbs and teeth and the ability to ruminate. Now, however, Cifelli⁵ has found no relationship between the relative diversities of both groups through time, and no evidence of competition or ordinal displacement.

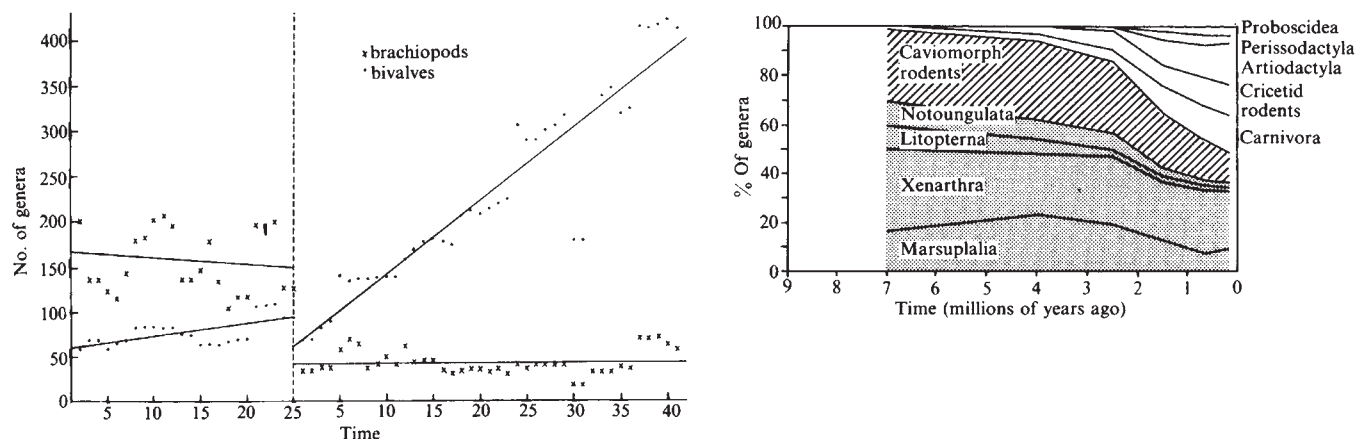


Fig. 1 (above left) Numbers of brachiopod genera and bivalve genera plotted against time. The time units correspond to geological stages and their length varies from about 5 to 10 Myr each. The vertical dashed line marks the Permo-Triassic boundary: the diversity of both groups was much reduced, but the bivalves recovered and radiated thereafter. The success of bivalves was probably not the result of continued attritive competition with the brachiopods. From ref. 3. **Fig. 2** (above right) Percentages of genera of different groups of mammals in South America over time. The shaded groups are endemic to South America, and the unshaded groups are typical of North America. The Panamanian land-bridge opened 3 Myr ago and North American invaders appear to have competed successfully with some of the typical South American natives. However, environmental changes caused many of the replacements. From ref. 9.

The most cited, and most studied, example supposedly indicating competitive replacement is the 'great American interchange'. Three million years ago, the Panamanian land-bridge appeared and permitted the previously isolated faunas of North and South America to intermingle⁶⁻⁹. Many native South American genera of mammals became extinct and were replaced by a slightly larger number of North American genera (Fig. 2). Certain South American groups (for example, Notoungulata, Litopterna, Xenarthra: large herbivores) were little affected, however, and North American invaders insinuated themselves into the faunas without causing extinctions. Environmental and climatic changes also tended to favour some North American groups. In addition, many South American mammals moved north and successfully established themselves in Central and North America. There is no clear evidence of the constant competitive superiority of one group over another.

A possible case of ongoing competitive replacement has been studied by Stanley and Newman^{10,11}. Balanoid barnacles compete successfully with chthamaloid barnacles on the rocky coasts of parts of northeastern Europe where their ranges overlap. The balanoids are also currently undergoing a broad adaptive radiation, while the chthamaloids are apparently on the decline, and the success of the balanoids is ascribed to their advanced wall structure and feeding mechanism. The fossil record is, however, poor and it cannot be demonstrated that there has been a reciprocal radiation of balanoids and decline of chthamaloids over time, and the relative present-day success of the two groups may be explained by selective predation¹². The evidence for competition as a major factor in this large-scale replacement is stronger than in most other cited examples, but it probably plays a

smaller part in the history of life than has been assumed.

Extinctions of large groups caused by physical factors and subsequent adaptive radiations can be seen in many cases in the fossil record. Further, it has become evident that there have been periods in the history of life during which numerous unrelated groups of plants and animals became extinct, apparently at the same time, and were replaced by new stocks. Among marine groups there have been four major extinction events¹³, during one of which, at the Permian-Triassic boundary, 225 Myr, over 90 per cent of shallow-water species died out. This has been explained by the reduction in continental shelf seas as the continents drifted together and fused into a single large land mass¹⁴.

Another major marine extinction event occurred at the Cretaceous-Tertiary boundary, 65 Myr, but this event is better known for the extinction of the dinosaurs and subsequent radiation of the mammals. Many hundreds of pages have been written about how the dinosaurs became extinct without our being any the wiser¹⁵⁻²⁰. As with all extinction and replacement events, the pattern must be established before the process can be determined. Questions still to be answered about the extinction of the dinosaurs include: how long did it take; did it occur at the same time everywhere; were dinosaurs already in decline; did dinosaurs become extinct at the same time as the profound extinctions amongst marine plankton; to what extent did climatic, or other, changes increase the relative fitness of the mammals; and was competition involved?

Explanations of purely 'competitive' replacements and of opportunistic adaptive radiations after major extinctions are deterministic. They assign relative fitness values to different groups in order to explain selective survival or extinction in

the face of changes in either the biotic or the physical environment. The alternative stochastic approach is probably applicable to many major replacements^{21,22}. In such cases, there is no single cause for the extinction but a whole range, including physical and biological factors, one or more for each species involved. Such explanations are probably valid more often than has been assumed, although they may seem unsatisfactory — an admission of no definite conclusion. They include, however, all cases between purely competitive take-overs and major extinction/opportunistic replacement events. Competition may increase the probability of extinction of a particular lineage, but it will rarely be the sole cause, whereas it could be postulated that a catastrophic change in the physical environment is sufficient on its own. □

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Solar structure

A bridge in a gap in solar oscillations

from Douglas Gough

THE science of helioseismology had its origins only eight years ago in the successful resolution by Deubner¹ of the spatial and temporal structure of the Sun's five-minute oscillations. By measuring Doppler shifts in solar spectra, Deubner was able to deduce the velocity structure of the Sun's visible surface. Depicted as functions of longitude and time the shifts displayed ridge-like features just as if waves were trapped in the outer, convecting layers of the Sun. The effect had already been investigated theoretically by Ulrich². Since then, many modes of oscillation have been observed and interpreted, not without controversy, and those reported by Duvall and Harvey³ in this issue of *Nature* (p.24) represent a significant extension to the collection.

The diagnostic value of Deubner's observations was appreciated immediately, and it was soon realized that the convection zones of contemporary theoretical solar models were too shallow. To correct them required an increase in the helium content. That in turn meant that the neutrino flux should be higher than previously calculated thus aggravating a conflict with observation which has now plagued astrophysicists for more than a decade.

The five-minute oscillations are an example of a class of oscillations called p modes. They were so named because pressure provides the predominant restoring force. Basically they are standing acoustic waves, modified by gravity. In addition there can be g modes, whose restoring force is buoyancy. These are internal gravity waves that can propagate only in convectively stable regions. They can exist in the solar atmosphere, or in the deep radiative interior of the Sun. Most interior gravity waves are difficult to observe, because the convection zone presents a severe potential barrier that almost isolates them from the Sun's surface.

Besides p and g modes there are also the so-called f modes. These can be regarded as the analogue of surface ocean waves, and have the remarkable property that if their wavelengths are much less than the radius of the Sun (or the depth of the ocean), their frequencies are independent of the density stratification of the Sun (or the ocean); their identification requires a knowledge of only the solar mass and radius.

Roughly speaking, discrete eigen frequencies of solar oscillation can be labelled by two independent integers: the order of the mode, $2n$, which measures the number of nodes along a radial line; and the degree l , which is the degree of the

spherical harmonic that factors from the eigenfunction. They correspond to the principal quantum number and the angular momentum quantum number in the theory of the hydrogen atom. The degree l can be regarded as a measure of the horizontal component of the oscillation wave number, and n characterizes the vertical component.

For any value of l , the modes can be ordered according to frequency. The frequencies of all g modes lie below the f-mode frequency ν_f at the same l , and decrease as n increases. The p-mode frequencies lie above ν_f , and increase with n .

Deubner's observations were designed to isolate nearly plane waves. Thus they were restricted to modes with $l \gg 1$, whose horizontal wavelengths are much less than the radius of the photosphere. Such modes are detectable for only comparatively low values of n . Hence the wave number vector, and also the velocity of propagation, are almost horizontal. Because temperature increases with depth, such waves suffer severe refraction and are thus confined within a shallow wave guide just beneath the photosphere. Its depth is $(2n+3)R/l$, where R is the radius of the Sun. The oscillation frequencies measure the mean sound speed in the wave guide, and thus provide a useful diagnostic of the Sun's structure. It was from this diagnostic that the high interior helium abundance Y , about 25 per cent by mass, was deduced. Notice, however, that because l is large (≥ 100), only the very outer layers of the Sun are sampled. Extrapolation to conditions in the solar interior depends on indirect deductions from the theory of stellar evolution, which at best can be regarded as somewhat uncertain.

A more direct indication of conditions in the solar interior has been obtained from measurements of either intensity fluctuations of Doppler shifts in light integrated over substantial portions of the solar image. There is strong cancellation from modes with $l \gg 1$, and only low-degree modes can be detected. As with the high-degree modes, most of the power is in oscillations with periods near five minutes, and since the fundamental acoustic pulsation period is about one hour, it follows that the total wave number must be quite large. Since l is small, n/l must be 'large' (typically $0 \leq n/l \leq 5$ and $15 \leq n \leq 30$). These modes are very interesting, because they propagate nearly vertically and so succeed in penetrating to the very core of the Sun.

Low-degree modes were first identified

by Isaak and his colleagues⁴ from Birmingham, and later were unambiguously resolved in the observations of Grec *et al.*⁵. A least-squares fit of the theoretical eigenfrequencies of a sequence of solar models to these new data⁶ was found also to favour $Y \approx 0.25$. However the fit is far from perfect. Consequently, by emphasizing certain aspects of only the low-degree data, it has been possible to maintain the view that, notwithstanding the high-degree modes, a solar model with $Y \leq 0.17$ will eventually turn out to be closer to the truth^{7,8}. This possibility has interesting cosmological consequences because the solar helium abundance imposes an important constraint on big-bang theories of primordial nucleosynthesis. The difference between a model with ($Y \leq 0.17$ and one with $Y \approx 0.25$ lies in the value of n that is assigned to a particular mode. That cannot be inferred from observations of only the low-degree five-minute modes, since the origin of n is not observed.

In this issue of *Nature* (see p. 24), an important paper³ appears to remove the ambiguity. By projecting longitudinally averaged Doppler measurements onto zonal harmonics, Duvall and Harvey have been able to connect the sequences of low-degree modes with those having $l \gg 1$. Since when $l \gg 1$ the f mode is unambiguously identified, subject only to the hypothesis that all the modes are excited (for which there is considerable justification), one can count ridges in the power spectrum at high l to identify n . Duvall and Harvey's connection with the low-degree modes then provides n for these modes too. The result corresponds to the prediction of the theoretical solar model with the higher helium content.

The matter must not be regarded as being totally settled, however. We must heed the discrepancy that still exists between theory and observation, not forgetting the problem with the neutrino flux. To resolve the structure of the Sun's interior will probably require knowing the frequencies of p modes with low n and l , and of some internal g modes too. Duvall and Harvey present observational evidence for the existence of those g modes. That gives us hope that more accurate diagnosis will some day be possible. □

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Malaria prevention

Oxidant killing of the malaria parasites

from F.E.G. Cox

THE continuing existence and spread of malaria coupled with an increasing incidence of drug resistance has encouraged those working in both immunology and chemotherapy to ask what exactly it is that kills malaria parasites in red blood cells. Clues and confusion abound but in the case of the rodent malaria parasites most widely used as models for human malaria, antibody seems to have little effect on *Plasmodium vinckei* and *P. chabaudi* that inhabit mature red blood cells whereas in infections with *P. berghei* and *P. yoelii* that preferentially invade reticulocytes antibody probably does play a part in parasite killing¹. The involvement of nonspecific factors in the killing of *P. vinckei* and *P. chabaudi* has been mooted for several years and the possibility that these factors are macrophage products has been championed by an Australian scientist, Ian Clark, and his colleagues². At the Fifth International Congress of Parasitology held in Toronto in August, Clark presented new data implicating toxic oxygen radicals in the killing of malaria parasites and the experimental evidence has just been published^{3,4} and supported by parallel studies in other laboratories^{5,6}.

Clark's approach has been direct. In his first series of experiments he injected alloxan, which generates reactive forms of oxygen as a result of redox cycling between alloxan and dialuric acid, into mice infected with *P. vinckei* and found that the parasitaemias fell from 30–60 per cent to less than 10 per cent within an hour and then continued to fall to very low levels after which further injections cured the normally lethal infections completely³. The decline in parasitaemia was not due to any diabetogenic effects of alloxan as previous injection of glucose did not reverse the effect whereas pretreatment with desferrioxamine a specific iron chelator that inhibits the formation of hydroxyl radicals, did. Similar, but less dramatic, effects were obtained with hydrogen peroxide and phenylhydrazine. In all the experiments, cure was accompanied by transient haemolysis and the intraerythrocytic death of the parasites. Organic hydroperoxides decompose to produce oxygen or peroxide radicals directly and in his second series of experiments Clark injected *t*-butyl hydroperoxide into mice infected with *P. vinckei* with results substantially similar to those obtained using alloxan⁴. Allison, using very small numbers of animals, also found that

repeated doses of *t*-butyl hydroperoxide had some adverse effects on another rodent malaria parasite, *P. yoelii*, when the initial parasitaemias were low but very little effect when at the levels used for *P. vinckei* in the experiments described above⁶.

Hydrogen peroxide has been implicated as a possible mechanism of malaria parasite killing by another group of scientists at the Middlesex Hospital Medical School in London⁵. In these experiments, four parasites were used — *P. chabaudi*, an avirulent line of *P. yoelii*, a virulent and lethal line of *P. yoelii* and *P. berghei*. The injection of hydrogen peroxide into infected mice rapidly reduced the parasitaemias in all cases except *P. berghei*. In *in vitro* experiments, hydrogen peroxide was found to kill the two parasites investigated — the avirulent *P. yoelii* and *P. berghei* — equally effectively.

These examples taken together indicate that oxygen or peroxide radicals or other derivatives of oxygen are involved in the killing of *P. vinckei*, *P. chabaudi* and a lethal line of *P. yoelii* (which invades mature erythrocytes) but not *P. berghei*. Whether this is due to the type of red cell invaded or to intrinsic factors in the parasites is not at all clear.

These experiments represent very artificial situations but if oxygen-mediated killing occurs during naturally resolving infections or after vaccination, what cells produce the reactive substances? Both the Australian³ and London⁵ scientists suggest that these are macrophages and that as infected blood cells squeeze between the fixed macrophages of the spleen and liver they trigger oxidative bursts and are subjected to oxygen products released by these cells. Evidence in support of this idea comes from other work with *P. berghei* in which it has been shown that peritoneal macrophages react with a chemiluminescent response, indicative of the generation of activated oxygen species, in the presence of whole or lysed parasitized erythrocytes⁷. On the other hand, during *P. berghei* infections in rats, the activity of superoxide dismutase (which removes superoxide) is reduced in the liver but normal elsewhere while catalase activity responsible for the breakdown of hydrogen peroxide is reduced in the liver but increased in the spleen⁸. The reduction of superoxide dismutase levels would lead to raised levels of oxygen radicals in the liver without the need to invoke any increased activity or parasite triggering. In the context of these experiments it is probably too simplistic to attribute comparable roles to the liver and spleen. It has also been suggested that cer-

tain natural killer cells may be the effector cells⁶ but no direct evidence has been offered to support this.

Although all these experiments relate to rodent malaria there is every indication that the same or similar processes may damage human parasites as *t*-butyl hydroperoxide inhibits the development of *P. falciparum* in culture⁴. If this is the case then there are a number of implications — there is a mass of evidence that shows that oxygen radicals are toxic⁹ and kill protozoa¹⁰ and malaria now joins the list; the pathology of malaria is commensurate with the production of toxic oxygen products³ or with a loss of superoxide dismutase⁸, and the possibility that infected cells are under oxygen stress and therefore more susceptible to the effects of oxygen radicals could explain the adaptive advantage of mutations, such as sickle cell trait, that allow certain individuals to resist malaria^{3,6}; and finally, it is possible that antimalarial drugs could operate by releasing oxygen radicals and could thus lead to a rational approach to the design of new drugs⁴. That immunity and chemotherapy should go hand in hand has always been a dream of parasitologists and there is every sign that this could now become a reality.

Caution must of course be paramount and it is a long way from mouse to man and we do not even know which species of rodent parasite most closely resembles the human form. The intimate relationship between protection and pathology should also provide grounds for concern. There will be many more studies in this exciting area and it will be some time before we know whether these recent experiments represent a solution or even a clue to the riddle of malaria parasite killing or whether they simply add to the confusion. □

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ERRATUM

In the article 'Increasing atmospheric methane' (301, 568; 17 February, 1983) the first eleven lines of the second column were accidentally transposed to the top of the third column. The section should have read:

The temperature rise is roughly 38 per cent of the increase calculated to have occurred as a result of increases in atmospheric CO₂. Furthermore, since methane plays a central role in the chemistry of the atmosphere the increase may have caused changes in the level of other atmospheric constituents such as OH, CO and O₃.

How can we explain the methane trends suggested by the data of Craig and Chou? What process or processes could have caused methane suddenly to increase towards the end of the sixteenth century after remaining constant for at least 25,000 years?

We apologise to the author, W.L. Chameides.

Palomar sky survey

Progress is not our most important product

from Virginia Trimble

ALL of us who are old enough to remember will be quite sure that many things are not as good today as they were thirty years ago — tomatoes, the post office, operatic tenors . . . The chief exceptions, of course, are the products of industrial research and development, which are all much better. Or are they? Mitchell Struble and Christ Ftaclas (*Publ. astr. Soc. Pac.* **94**, 763; 1982) of the University of Pennsylvania report that recent prints of the Palomar Observatory Sky Survey (POSS) contain less information than the older prints and that the chief cause is graininess and specular reflection in the new photographic paper.

The POSS consists of 1,870 plates taken on the 48-inch Schmidt telescope from 1949 to 1958, and covers the sky north of -30° declination to a limiting stellar magnitude of about 21 (at the blue end of the spectrum) and 20 (at the red end). A similar survey of the southern sky, being carried out by the UK Schmidt Telescope Unit and the European Southern Observatory, and a second epoch survey of the northern sky

from Palomar are now in progress. The POSS is a major astronomical tool, used in the identification of radio and X-ray sources; selection of candidate objects for detailed studies; investigations of the morphology of nebulae, galaxies and clusters; and many other purposes.

The first paper print copies of the POSS were issued in 1954 on Eastman Kodak unicontrast double-weight paper. There have been several subsequent reprintings from the original glass plates, the most recent few on Polycontrast Rapid II RC paper, initially called Ektabrome SC and recognizable by multiple impressions of the words 'this paper manufactured by Kodak' on the reverse side (it also feels rather like plastic).

Struble and Ftaclas have studied images

of galaxies of varying angular size on both old and new prints (and, for comparison, on one of the much rarer sets of glass copies of the Survey). They find, on average, that images on the new prints are smaller (the outer regions of the galaxies have been lost) and rounder (that is, there has been some smearing or loss of angular resolution, equivalent to poor seeing) than those on the old prints. The effects are most conspicuous for the smallest images and are sufficient to distort properties of galaxies often measured in morphological studies using the POSS.

I happen to have old and new blue prints of the field including the Crab Nebula and would describe the new print (under $\times 10$ magnification) as looking softer than the old one. For instance, fewer of the discrete filaments near the edge of the Nebula can be resolved from the continuous emission. This would be a blessing in passport photographs for those of us who remember the tomatoes of thirty years ago. Its effects on assorted applications of the POSS deserve further study. □

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100 years ago

CENTRAL AND WEST AFRICA

THE brilliant journey of Major Serpa Pinto across Africa from Loanda, by the Zambesi to Natal, must be fresh in the recollection of our readers. The present narrative may be regarded



FIG. 1.—A Muata of the T'chiboco.

as complementary of the major's exciting story. Captains Capello and Ivens were member sof the original expedition along with Major Pinto, and for the first part of the journey the three companions worked together. The object of the expedition, which was organised by the Portuguese Government, was to thoroughly survey the great artery which — a tributary of the Congo — runs from south to north between 17° and 19° E. of Greenwich, and is known as

the Cuango, as also to determine all the geographical bearings between that river and the west coast, and make a comparative survey of the hydrographical basins of the Congo and Zambesi.

The country traversed is mostly mountainous, cut up by innumerable streams and valleys, rich in many parts in vegetation,

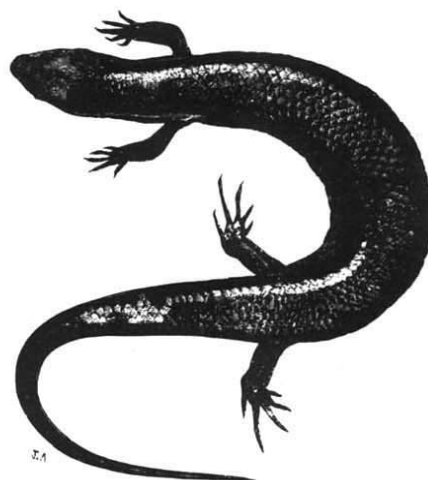


FIG. 5.—Euprepes Ivensi (new species), River Cuango.

and even in metals, and having a considerable population clustered in villages, each of which is ruled by its chief. With each of these chiefs much diplomacy had to be used in order that the explorers and their followers might obtain provisions and be allowed to pass. Among the people of this region we find the same elaborate methods of dressing the hair, so common in Central and Western Africa, and with which readers of recent African travel must be familiar. We have some interesting details as to the history of some of the leading tribes of the

region, from which it is evident that for centuries the various African peoples have been in a state of almost constant migration, that the so-called states are exceedingly unstable, and that even here it would be hazardous to regard any one race as unmixed. We give here two types: Fig. 1, a Muata, or ruler, of the T'chiboco; and Fig. 2, a woman of Cangombe.

The sources of the Cuango were found at a height of 4756 feet, at about $11\frac{1}{2}^\circ$ S., and a little



FIG. 2.—Woman of Cangombe.

east of 19° E., in one of the most extraordinary watersheds to be met with anywhere. It is thus described:

"An extensive tract of land, all hill and dale, marks this culminating point, a sort of St. Gothard of the African waters. On the north, running through a narrow and tortuous valley, appeared the Cuango, which, shortly after its birth, flows at the foot of the plantations of manioc and massanmaba, growing abundantly upon the slopes, and at that time filled with girls and women engaged in hoeing and other field labours".

The oncogenic potential of herpes simplex viruses: evidence for a 'hit-and-run' mechanism

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Experiments to determine the mechanism of transformation of herpes simplex virus (HSV) have identified fragments of viral DNA which are able to initiate transformation. No set of viral genes seems to be consistently retained or expressed in the transformed cells or in human cervical tumours, suggesting that viral DNA is not needed to maintain the transformed phenotype. In fact there is no conclusive evidence that initiation of neoplasia is mediated by a viral protein. Here we revisit the 'hit-and-run' hypothesis and its implications for HSV-induced tumorigenicity.

THE recent surge in interest in the two herpes simplex viruses (HSV-1 and HSV-2) stems not only from the fact that genital infections with these viruses have reached epidemic proportions, and that infection is associated with an increased risk of cancer, but also because virologists are now able to study these problems at the molecular level. The suggestion that HSV-2 is involved in the aetiology of squamous cell carcinoma of the uterine cervix was initially based on serological and epidemiological studies¹⁻⁴. Supporting evidence came from finding viral antigens expressed in tumour cells⁵⁻⁸, as well as the detection of viral RNA^{9,10} and DNA¹¹ in some tumours. In addition, HSV-1 and HSV-2 are able to transform rodent cells to a malignant phenotype^{12,13}. In the past few years several laboratories have concentrated on identifying the viral genes responsible for morphological transformation and the viral products that persist in transformed and tumour cells. It is clear that exposure to specific sequences of HSV DNA can produce changes in rodent cells that cause them to be tumorigenic. Also, certain regions of the genome are frequently expressed and translated into recognizable viral antigens in both experimentally transformed cells and in human cervical carcinoma cells. However, although these viral footprints can be catalogued there is no indication that the continued expression of any particular herpesvirus antigen is necessary to maintain the transformed state nor that certain sequences of HSV DNA are always retained or expressed.

In fact there is growing evidence that cells can remain transformed in the absence of detectable herpesvirus sequences and that a protein may not be needed even to initiate transformation. These observations greatly strengthen the hypothesis that herpes simplex virus acts in a 'hit-and-run' manner¹⁴⁻¹⁶ to induce the changes in cells which cause them to be tumorigenic. Also, they suggest that the search for tumour-associated antigens, which has proven fruitful in unravelling the biology of the smaller DNA tumour viruses, may not be an appropriate pathway for understanding the basis of HSV-induced tumorigenicity.

Transforming genes

Two approaches have been taken to identify the viral genes responsible for transformation. In the first, cells transformed by whole, inactivated HSV genomes were examined for persistent viral products. Nucleic acid hybridization often detected HSV RNA^{17,18} and DNA^{19,20}. The quantity and complexity of sequences changed with passage of the cell lines but in general very few copies of just a portion of the HSV genome were present. Nevertheless, experiments with temperature-sensitive (ts) mutants²¹ showed that the resident viral sequences could be mapped throughout the HSV genome. In some hybridization studies, however, no viral sequences were detected in the transformed cell lines²², a failure attributed to the insensitivity of

using a large viral DNA probe to detect small fragments of HSV DNA^{19,20}.

Several HSV-specific antigens have been reported in transformed cells including membrane glycoproteins^{13,23-25}, non-structural proteins VP143 (ref. 26), thymidine kinase²⁷, ICP10 (ref. 28) and several other polypeptides with presumed viral counterparts²⁹. Although some of the proteins, including glycoprotein A/B and VP143, were frequently found, no polypeptide was invariably expressed in all HSV transformants. The proteins expressed in cells transformed by UV-inactivated HSV and the viral DNA retained by these cells implicated sequences located around 0.30–0.41 and 0.58–0.63 map units on the HSV genome as having some role in transformation. However, these types of analyses did not fully define the sequences needed for transformation, nor suggest a mechanism. Due to the lack of well defined transformation-deficient mutants of HSV, it has not been possible to test critically whether any of the proteins associated with HSV transformation has an essential role. It remains possible that the expression of these proteins is fortuitous and that those proteins that are frequently expressed either confer a growth advantage on the cells or are close to, and therefore fortuitously expressed with, sequences that are important in transformation.

The second approach used to identify the genes needed for transformation involved using defined fragments of viral DNA to transform rodent cells. These experiments gave unexpected results. First, although transforming genes for both HSV-1 and HSV-2 could be identified they were not in an homologous position on the two genomes; a rather surprising finding in view of the fact that almost all genes are arranged colinearly along these genomes. Second, two different fragments of HSV-2 DNA could transform rodent cells depending on the assay used to produce the transformants. Third, the transformed cells did not always retain any of the viral sequences used to initiate transformation. The experimental support for these three conclusions is worth summarizing.

Three laboratories have located a fragment of HSV-1 DNA between 0.31 and 0.42 map units that is able to transform hamster embryo cells and BALB 3T3 cells (refs 25, 30 and P. Schaffer, personal communication). The fragment encodes proteins, for example glycoprotein A/B and VP143, that have been found in transformed cells, and sequences from the region have been shown to persist in some cells transformed by UV-inactivated virus²⁰. Nevertheless, attempts to detect viral sequences in cells transformed by the HSV-1 fragment have been unsuccessful (ref. 30 and P. Spear, personal communication).

Two different fragments of HSV-2 DNA, neither of which is homologous to the HSV-1 fragment, have been found to transform rodent cells. One is the Bg/II N fragment of HSV-2 (333) which maps between positions 0.58 and 0.63 and has

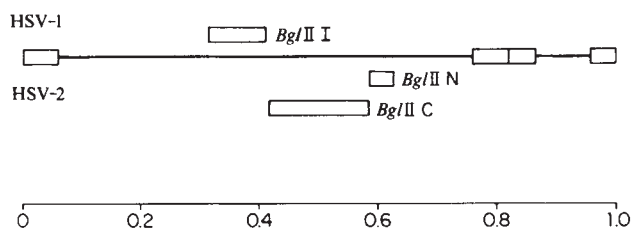


Fig. 1 The location of transforming genes along the HSV genome. The blocks indicate the position of restriction enzyme fragments of HSV-1 (above) and HSV-2 (below) transforming DNA along the prototype arrangement of the HSV genome. The numbers are map units.

been used to alter the growth properties of hamster and rat embryo cells and BALB and NIH 3T3 cells^{30,31}. The method of selection was focus formation in low serum or colony formation in semisolid medium. Cells transformed in these conditions were stable, tumorigenic and generally retained some sequences of viral DNA but no viral proteins were found. However, an alternative assay—the continuous passage of hamster embryo cells after exposure to fragments of HSV-2 DNA—identified a different transforming fragment of HSV-2, the *Bgl*II C fragment which maps between 0.43 and 0.58 (ref. 28). Two transforming functions within the *Bgl*II C fragment could be distinguished by the fact that sequences from the left-hand end of the fragment were able to immortalize normal diploid hamster cells but the conversion to tumorigenicity required DNA from both the left and right ends of the *Bgl*II C fragment (R. J. Jarriwalla, personal communication). Although the transformed cells seem to retain viral DNA, it is difficult to assess this result as some homology exists between *Bgl*II C viral sequences and normal rodent DNA.

Taken together, these experiments indicate that the herpes simplex genome may encode several transforming genes (see Fig. 1) which are used differently in various selection procedures, and that the genes may have diverged between the two viruses.

Further experiments to define more precisely the transforming sequences of the *Bgl*II N fragment suggested that an unusual type of event, not previously described for a DNA tumour virus, was taking place. When the 7.6-kilobase (kb) *Bgl*II N fragment was cleaved with various restriction enzymes and the individual fragments used in transformation assays, the transforming region was located to a 2.1-kb fragment bounded on the left by a *Bam*HI site which is 0.55 kb from the *Bgl*II site, and on the right by a *Pst*I site (see Fig. 2). Cells which were transformed by the complete *Bgl*II N fragment, however, did not retain these sequences (but instead retained viral DNA from the right-hand end of the *Bgl*II N fragment); nor were they to be found in any of three cell lines which had been transformed by the 2.1-kb fragment from the left-hand end of *Bgl*II N. Whether the loss of these sequences is obligatory or not is unknown. It is clear, however, that sequences of HSV-2 DNA that are able to transform cells *in vitro* need not persist in the transformant.

To determine whether any protein encoded by the 2.1-kb sequence is needed to initiate the transforming event, we first identified the proteins by *in vitro* translation of hybrid-selected mRNA³². Although this technique identified three proteins, characterized by a molecular weight (MW) of 140,000 (140K), 61K and 35–38K, none of them seems to be wholly encoded within the transforming fragment. In the case of both the 140K and 61K protein, the 5' end of the transcribed RNA came from well outside the transforming fragment. For the 35–38K protein, which is synthesized early before the onset of viral DNA replication and continues to be made late in infection, DNA sequence analysis (D.A.G., S. Weinheimer and M. Swain, in preparation) of the transforming region has located precisely the putative coding region of the gene and its regulatory sequences. Clearly, the entire message for the protein is transcribed

from sequences within the left-hand end of the *Bgl*II N fragment, however the sequences involved in transcriptional control—the 'TATAA' and 'CAT' boxes—as well as the first two nucleotides of the ATG 'start' site, are localized within the 0.55 kb between the *Bgl*II and *Bam*HI sites, a region which is not needed for transformation (see Fig. 2). As the transcriptional control sequences of the 35–38K protein are not present and have been replaced by bacterial sequences in the cloned 2.1-kb transforming fragment, it is unlikely that this protein is essential for transformation. Although transcription could begin from within plasmid sequences or from cellular sequences following integration, one would have to argue that this unusual form of transcription occurred to synthesize a protein needed for transformation initiation, and that event was followed by the loss of the DNA encoding the 38K protein. Formally this possibility exists but certainly invokes a complicated mechanism. Although a 35–38K protein has been reported in HSV-2-transformed hamster cells^{29,33}, we have been unable to detect it in any transformed cell line using two specific monoclonal antibodies³². More intensive analysis is needed to exclude the possibility that the 2.1-kb fragment contains a still unidentified open reading frame coding for a minor, undetected protein. For now, however, it seems appropriate to suggest that one possible mechanism for HSV-2-mediated transformation does not involve a herpesviral protein and that sequences of the viral DNA need not persist in transformed cells.

Role of HSV in cervical cancer

The epidemiology of cervical neoplasia has been extensively studied, and is a topic this review will not attempt to cover. Almost all the sero-epidemiological investigations have taken the form of case-control studies in which the prevalence of HSV-2 antibodies and/or antigens among women with cervical neoplasia was compared with the prevalence in women without such lesions. In general the studies conclude that HSV infections, not just promiscuity or other venereal diseases, can be linked to cervical intra-epithelial neoplasia although not to all forms of cervical neoplasia or dysplasia. These results suggest that HSV may have a unique interaction with the squamous epithelial cells of the transformation zone, the site from which almost all cervical squamous neoplasms arise (for review see ref. 34). For heuristic reasons it is convenient to consider two hypotheses: either HSV acts as an initiator of transformation in susceptible target cells and a second step commits the cells to become neoplastic, or other factors predispose the cells towards neoplasia and infection with HSV is the final step. Experiments to decide between the two alternatives take the form of an archaeological dig to recreate the initial event from the remaining fragments of viral information.

Early studies^{35,36} which were unable to detect HSV-2 nucleic acids in tissue from cervical biopsies were plagued with questions about the sensitivity of the assay. More recently, several laboratories have reported the detection of HSV-specific RNA in cervical cells by *in situ* hybridization^{9,10,37,38} with the numbers of positive tumours ranging from 35% (ref. 9) to 67% (ref. 37). That less than 100% of the cases were positive suggests that either there is more than one aetiology for cervical carcinoma, or that in some cases viral nucleic acids can be lost entirely. When the RNA transcripts expressed in the tumour cells were mapped by *in situ* hybridization using fragments of HSV-2 DNA, the results indicated that transcription was limited and came from three blocks of sequences located at map positions 0.07–0.40, 0.58–0.63 and 0.82–0.85 (ref. 38), two of which regions have been implicated in transformation *in vitro*. However, no one sequence was invariably expressed in all the HSV-positive specimens thus it was impossible to identify a tumour-specific gene. In preliminary experiments we found HSV DNA in three of nine invasive cervical tumours by Southern blot hybridization using as probes fragments of the HSV genome (J.K.M. *et al.*, unpublished). Of the three tumours, one was positive only with the *Bgl*II N (0.58–0.63) probe;

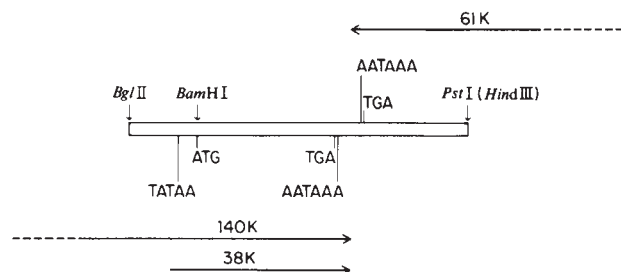


Fig. 2 The location of genes encoded by the left-hand end of the HSV-2 *Bgl/II* N fragment. The lines with arrows indicate the direction of transcription of three mRNAs which can be selected by hybridization to the left-hand end of the *Bgl/II* N fragment and the molecular weights of the proteins they encode. DNA sequence analysis of the fragment has localized the 5' transcriptional regulatory sequence (TATAA) for the 35-38K protein between the *Bgl/II* and *Bam/II* sites and the termination codon (TGA) and the polyadenylation signal (AATAAA) approximately 1 kb downstream from the initiation codon (ATG). On the opposite strand putative signals for the termination (TGA) and polyadenylation (AATAAA) of the 61K protein were found. Sequences coding for the 140K protein have not yet been found.

another was positive only with the *Bgl/II* J (0.32-0.40) probe; and the third was positive with both probes. Again the necessary persistence of any specific sequence of HSV-2 DNA is questionable.

There is also some evidence that sera from cervical carcinoma patients contain antibodies to HSV proteins and that viral antigens can be detected in cervical squamous cell carcinomas. One of the most extensively described of these is AG4 (or ICP10), reportedly a HSV-2 immediate early protein with a molecular weight of 160K, which is expressed on 80% of tumour cells and to which patients have complement-fixing antibody³⁹. This protein is also expressed in cells transformed by the *Bgl/II* C fragment²⁸, however, the viral sequences encoding this polypeptide have not been identified. Other antigens which have been demonstrated frequently on cervical tumour cells^{7,8,38} react with antisera directed against the major HSV-2 DNA-binding proteins, ICSP 11/12 and ICSP 34/35. The former has also been demonstrated in cells transformed by UV-inactivated HSV-2^{24,26}, and is encoded within sequences which have transforming activity from the HSV-1 genome. Although these findings are tantalizing, it is possible that the only function of these proteins is to confer some growth advantage without being necessary for the initiation or maintenance of the transformed state.

It continues to be difficult to prove that infection with HSV is the first step towards cervical carcinoma. One could argue that neoplastic changes alter the susceptibility of the host to viral infections and that HSV infections and antibodies follow, rather than precede, malignant transformation but there is evidence that changes in the squamous epithelium do not alter virus susceptibility⁴⁰. Interestingly, papillomavirus DNA has recently been demonstrated in several cervical carcinomas (L. Gissman and H. zur Hausen, personal communication) and in the case of laryngeal papillomatosis it has been shown that papilloma virus can persist for many years in benign tumours, which can convert to malignancy after X-ray irradiation. Might HSV and human papilloma virus act synergistically? If so, does HSV act as the initiator or the effector of malignant transformation?

Hit-and-run mechanisms

The long history of viral oncogenesis has pointed to the acquisition of viral genes as the factor that converts normal cells and maintains them in a malignant state. Evidence for a maintenance function for viral genes came from studies with ts mutants of papovaviruses⁴¹ and retroviruses⁴² in which the transformed phenotype was associated with the continued expression of a viral gene. Such evidence never existed for adenoviruses or

herpesviruses but by analogy with the smaller viruses it was frequently presumed that all the DNA viruses would be similar. Therefore, a role for HSV in the initiation of transformation but not maintenance was proposed¹⁴⁻¹⁶, but not widely held. More recently, the discovery of cellular oncogenes (*c-onc*) has focused attention on cellular genes that can mediate oncogenesis, once the cell has undergone an initiation event. One important question is whether cellular oncogenes can be induced by the expression or insertion of herpesvirus sequences. If so, cervical carcinoma could be similar to bursal lymphoma, in which sequences from the avian leukosis virus in some way promote the expression of the *c-onc* gene, *c-myc*^{43,44}. Perhaps a short stretch of nucleotides from within the *Bgl/II* N fragment, not detected by blot hybridizations using the entire fragment as a probe, inserts itself into cellular DNA in the vicinity of a *c-onc* and promotes expression in transformed cells and human tumours. Transformation mediated by other HSV DNA fragments could either have the same cause or be the result of a protein encoded by the viral sequences which turned on the expression of a *c-onc*. It has been demonstrated previously that transformation by HSV stimulates the production of C-type viruses^{15,45} and that the interaction of the two viruses might have a co-carcinogenic role, but this phenomenon has not been demonstrated in NIH 3T3 cells transformed by the *Bgl/II* N fragment³¹, nor have any of the previously described transforming sequences of HSV been shown to be able to activate retroviruses⁴⁶.

Another possible explanation for the unusual tumorigenic potential of HSV is that it acts as a mutagen. All the human herpesviruses induce chromosomal aberrations. Both random damage and specific gaps have been reported in HSV-infected cells^{47,48} as well as the fact that an early viral function is responsible for these events^{49,50}. Recent experiments⁵¹ demonstrating the mutagenic activity of HSV-1 by measuring the rate of mutation at the HGPRT locus in cells exposed to UV or neutral red-inactivated HSV-1, have found that HSV is as potent a mutagen as the chemical carcinogen 4-nitroquinoline-1-oxide. It has also been shown (J. Schlehofer and H. zur Hausen, personal communication) that, like chemical and physical carcinogens⁵², HSV can mediate gene amplification in simian virus 40 (SV40)-transformed cells. Interestingly, viral DNA could not be detected in the mutagenized cells suggesting that mutation does not occur by the insertion of large stretches of HSV DNA into a gene, nor does a viral gene confer the new phenotype. Of great importance will be studies which identify the viral genes involved in HSV-induced mutagenicity. Prime candidates include viral-encoded enzymes that degrade DNA, for example, alkaline exonuclease or the exonuclease activity associated with the DNA polymerase, as well as enzymes involved in DNA biosynthesis, for example, DNA polymerase, ribonucleotide reductase or thymidine kinase. Another possibility is that transformation is caused directly by sequences of HSV DNA that are particularly active in recombination into, and excision from, the cellular genome. If transformation could be explained by active recombination of HSV sequences into the host cell chromosomes, the failure to detect viral DNA could be due to either rapid excision of those sequences, or rearrangement of viral DNA such that it does not remain in an homologous position in all cells. It will be interesting to determine whether the sequences of HSV DNA that can cause mutations can also transform cells to a malignant phenotype.

So far, support for a hit-and-run mechanism of HSV-mediated transformation has come from negative evidence; the failure to detect a consistent set of viral sequences or a specific protein in transformed cells or human tumours. The viral footprints are circumstantial evidence that HSV has been at the scene of the crime and was probably responsible for the damage. The recent identification of sequences of HSV DNA that can transform cells in tissue culture without persisting, or perhaps without even encoding a viral gene product, has provided a next step in precisely defining how a hit-and-run mechanism might occur. We hope that future experiments will provide

positive data to explain the action of herpesviruses in experimentally induced transformation and establish the elusive link between HSV and human malignancy.

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ARTICLES

Observations of solar oscillations of low and intermediate degree

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Measurements are presented of solar velocity oscillations with spherical harmonic degree 1-139 and angular order ~0. With an amplitude sensitivity of ~2 cm s⁻¹, trapped acoustic wave modes of radial orders 2-26 are observed at frequencies between 1.7 and 5.5 mHz. The radial order identifications of low-degree modes previously inferred from theory are confirmed. Only marginal evidence of long-period, gravity-mode oscillations is found.

THERE is great interest in probing observationally the solar interior to test and improve the theory of stellar interiors, to improve understanding of the underlying basic physics, and to help clarify processes in the convective interior of the Sun such as those that are believed to produce the solar activity cycle. Several methods are available to probe the solar interior. For example, various modes of wave oscillations are trapped in cavities within the Sun. The frequencies of oscillation are sensitive to interior conditions and thereby provide a kind of seismic probe. Our work is aimed at an accurate determination of the frequencies of modes that cover a wide range of depth. First, we investigate which oscillations are the most useful to observe for probing a large depth range.

Solar oscillations having a ~5-min period arise from acoustic waves trapped in a cavity bounded above by the rapid change of density near the surface and below by the increase of sound speed with depth. The radius, r , of the lower boundary of the cavity depends on the spherical harmonic degree, l , the local sound speed, c , and the frequency of the oscillation, ν . Given the local sound speed at a radius inside the Sun, an adequate approximation of the value of r may be found as follows. The

dispersion relation for horizontally propagating acoustic waves in spherical geometry is¹

$$4\pi^2\nu^2 = l(l+1)\frac{c^2}{r^2} \quad (1)$$

This may be transformed to

$$l = -\frac{1}{2} + \left(\frac{1}{4} + \frac{4\pi^2\nu^2 r^2}{c^2} \right)^{1/2} \quad (2)$$

Figure 1 shows the lower boundary for 3-mHz oscillations as a function of degree, computed from equation (2) with sound speeds derived from a compilation of solar models². Except for small leakage effects, the frequency of a given mode of oscillation is not affected by the structure of the Sun inside the lower boundary of the cavity for that mode. Thus, in principle, resolution of the radial variation of solar structure can be obtained by sufficiently precise observations of oscillation mode frequencies having different degrees.

Measurements of solar oscillations with degree 0-3 in the frequency range 1.8-5.1 mHz have been made using integral

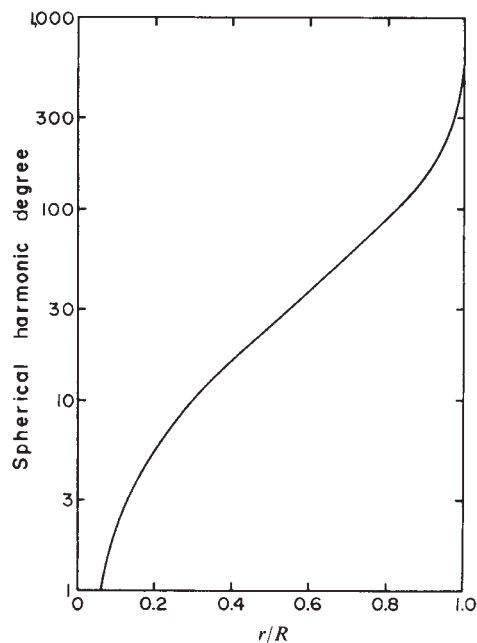


Fig. 1 Solar oscillations of frequency 3 mHz are trapped in a cavity whose outer boundary is close to the surface and whose inner boundary is approximated by the curve as a function of fractional solar radius r/R . Acoustic oscillation modes of spherical-harmonic degree <140 probe most of the depth range of the Sun.

(full disk) sunlight³⁻⁶. By using a masked solar image, oscillations with degree 3–5 have been observed between 2.5 and 4.2 mHz (refs 7, 8). Well-resolved solar images have been used to define the spectrum of 5-min oscillations having degree from ~ 140 to $\sim 1,000$ (refs 9–11). Figure 1 shows that these previous observations provide depth resolution over roughly the inner 20% and outer 10% of the solar radius. The previously unobserved degree range from 6 to 140 covers 70% of the radius. Observations of this region of the spectrum of 5-min solar oscillations should be the most valuable in resolving the major part of the depth variation of solar structure and dynamics¹². For that reason we have started a series of observational studies of solar oscillations of low to intermediate degree.

In collaboration with M. Pomerantz, we made observations from the geographic South Pole to define the 5-min intensity oscillation spectrum for degrees <140 (ref. 13 and J. H., T. D. Jr and M. Pomerantz, in preparation) with degree resolution of ~ 3 , for angular orders m from 0 to l , and with frequency resolution of $\sim 0.5 \mu\text{Hz}$. The reduction and analysis of these observations is not finished and because of current interest, we present here results from our test observations of radial velocity oscillations made with the McMath Telescope at Kitt Peak National Observatory before the South Pole observations.

Observing single modes of oscillation

We used observational techniques developed for measurement of oscillations with degree >140 with only a change of solar imaging (J.H., T.D. Jr and E. J. Rhodes, in preparation). There are 19,600 separate spherical harmonics with degree <140 . A full solar disk image recorded with ~ 200 resolution elements in two dimensions allows all these harmonics to be measured with only a small amount of crosstalk caused by our inability to observe the back side of the Sun. This approach was used for our South Pole observations, but for the present results we observed a one-dimensional solar image produced by a 1-m focal-length cylindrical lens. By using the lens to average the Sun's image in a direction perpendicular to its rotation axis, we greatly reduced the amount of data to be recorded by effectively eliminating high-angular-order spherical harmonics to leave mainly zonal harmonics of angular order 0. This process for eliminating non-zonal harmonics is not perfect because of unequal weighting across the solar disk owing to limb darkening

Table 1 Relative amplitude response of the observation and reduction method for $l = 40$, $m = 0$ to neighbouring modes (blanks indicate null response)

l	36	37	38	39	40	41	42	43	44
m									
0	4		-55		100		-55		3
1		20		-74		76		-20	
2	-2		29		-55		31		-2
3		-5		19		-21		6	
4	0		-2		4		-3		0

and the centre-to-limb variation of the line-of-sight component of radial velocity fluctuations. To help unravel the observations, we computed the sensitivity of this process to neighbouring harmonics by averaging, in the same way as our observations, normalized spherical harmonic functions of degrees 36–44 and angular orders 0–4 weighted by limb-darkening and line-of-sight projection effects. These averages were then multiplied by the normalized Legendre function of degree 40 and integrated to produce the results in Table 1.

These results show a considerable amount of crosstalk between neighbouring degrees and angular orders. For example, a $l = 39$, $m = 1$ mode would appear at 74% of its true amplitude in our $l = 40$, $m = 0$ analysis. Similar confusion applies to other degrees. However, sensitivity to a few angular orders $\neq 0$ actually proved to be a considerable aid in identifying various spectral features.

To observe Doppler velocities, we focused the one-dimensional solar image on the slit of a 13.7-m spectrograph equipped with a two-dimensional diode array camera. About 200 picture elements covered the north–south direction of the east–west averaged solar image and 80 picture elements covered a portion of the optical spectrum. We observed the solar Ni I line at 631.4668 nm, a photospheric line of moderate strength that is relatively free from blending. Observations were made on 17–18 March 1981 and 4–7 June 1981 at a cadence of one recording per minute for an average duration of 7.8 h each day.

The Doppler shift of the Ni I line was determined at each spatial position in each spectrum recording. After various registration and slow trend removal processes (which eliminated information of degree zero), we reduced each day's observations to an array of Doppler velocities as functions of space and time. We transformed the spatial information by multiplying the data by normalized Legendre functions of degree 0–139 and then integrating the results to produce a time series of 140 spherical harmonic coefficients. The normalization of the Legendre functions $P_l(\mu)$ was chosen so that

$$\int_{-1}^1 [P_l(\mu)]^2 d\mu = 2 \quad (3)$$

where μ is the cosine of the colatitude. Spherical harmonic coefficients, a_l , follow from

$$a_l = \int_{-1}^1 vel(\mu) P_l(\mu) d\mu \quad (4)$$

where vel is the observed velocity signal. The coefficients were then Fourier transformed in the time domain to produce $l - \nu$ amplitude spectra such as Fig. 2. We now consider the 4–7 June 1981 observations reduced as a single, interrupted time series of 74 h duration. In this spectrum the noise level in a single spectral element corresponds to a single-frequency a_l coefficient with an amplitude of $\sim 2 \text{ cm s}^{-1}$.

Spectrum of 5-min oscillations

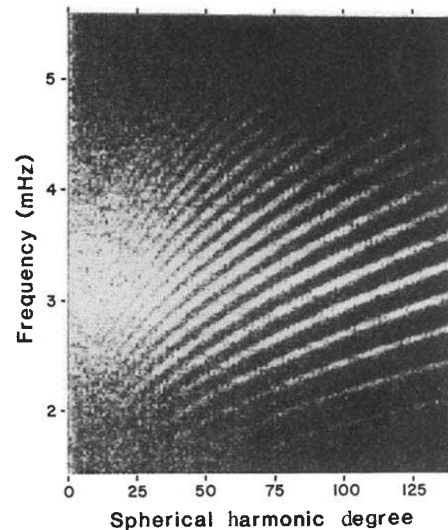
At degrees >16 , the spectrum is characterized by isolated ridges of signal that correspond to trapped oscillations with radial order numbers, n , from 2 to 24. The ridges appear above the noise level for frequencies between 1.7 and 5.5 mHz and are strongest at $\sim 3.2 \text{ mHz}$. The 4–5 degree width of the ridges is consistent with the values of Table 1. The mode frequencies at high degree match previous measurements and we have adopted

Table 2 Coefficients for splines that match the observed frequencies (μHz)

n	l_a	ν_a	l_b	ν_b	l_c	ν_c	f_a	f_c	Points
10	13	2,129.2	49	3,026.8	71	3,411.8	-0.710	-0.095	40
11	16	2,372.3	33	2,858.3	45	3,125.5	-0.429	-0.246	26
12	6	2,141.1	13	2,411.2	45	3,299.6	-1.901	-0.347	32
13	6	2,280.7	13	2,551.5	45	3,471.3	-1.496	-0.365	32
14	4	2,325.3	15	2,764.0	43	3,594.7	-1.422	-0.290	32
15	2	2,353.9	12	2,794.8	36	3,571.2	-2.646	-0.251	28
16	1	2,424.4	9	2,814.2	42	3,895.4	-4.969	-0.178	28
17	1	2,560.1	8	2,910.8	27	3,601.5	-4.786	-0.185	23
18	1	2,693.5	9	3,090.5	24	3,651.8	-5.035	-0.415	20
19	1	2,828.6	10	3,270.9	23	3,763.0	-4.227	-0.222	17

Table 3 Frequencies of selected low-degree oscillations (μHz)

l	1	2	3	4	5	6	8	10	14	20
11								2,163	2,306	2,499
12						2,143		2,303	2,446	2,645
13				2,190		2,280		2,441	2,586	2,790
14				2,324	2,371	2,415	2,498	2,580	2,726	2,937
15		2,352	2,407	2,458	2,506	2,549	2,636	2,718	2,869	3,081
16		2,486	2,541		2,641	2,685	2,774	2,854	3,010	3,226
17	2,560	2,620	2,675			2,823	2,911	2,995	3,151	3,371
18	2,692	2,756	2,812	2,865	2,914	2,960	3,049	3,133	3,292	3,514
19	2,827	2,890	2,947	3,000	3,050	3,097	3,187	3,271	3,431	3,657
20	2,964	3,024		3,147	3,186	3,233	3,324	3,408		3,800
21	3,099	3,161		3,272	3,324	3,370	3,462	3,550	3,712	3,943
22	3,233	3,295	3,351	3,406	3,463	3,508	3,601			4,086
23	3,367	3,430	3,489	3,545	3,597	3,644		3,829		
24	3,505	3,566		3,682	3,732	3,784				
25		3,701		3,819						
26		3,840								

**Fig. 2** Spectrum of solar oscillations in the degree range 0–139 and the frequency range 1,416 to 5,583 μHz . The frequency resolution has been degraded to 8 μHz .

the radial order numbers established for high degree observations. At degrees 1 and 2 the spectrum shows signals at frequencies that have previously been identified with low-degree oscillations having radial order numbers from 15 to 26. The assignment of radial order number at low degree has previously been done by comparison with theory. Our spectrum confirms the assignment on purely observational grounds.

For degrees between ~ 3 and ~ 16 the sensitivity of our observations to 5 degrees at once, spectral sidelobes at $\pm 11.6 \mu\text{Hz}$ caused by daily interruptions and the intrinsic crowding of spectral features all produce an apparently confused spectrum. To assist in the correct identification of the multitude of spectral features we constructed *echelle* diagrams for each radial order so that the ridge of signal is displayed as a straight up-and-down feature as a function of degree. That is, the steps are degree and the base frequency of each step is shifted so that the spectral features with a specified degree, angular and radial order fall along a vertical line. Our diagrams are similar to the *echelle* diagrams introduced by Grec *et al.*⁶ except their steps are radial order rather than degree. Their diagrams are most useful at low degree where the frequency spacing between modes as a function of radial order number is nearly constant. The increasingly varying spacing between modes at higher degree, together with our ability to resolve oscillations of different degree, led us to adopt the new scheme. To make an *echelle* diagram, we need nearly correct frequencies for modes of a given radial order as a function of degree. Approximate frequencies were estimated and power series expansions computed to allow construction of preliminary diagrams. These were then used to identify specific modes with good frequency resolution. Final frequency assignments and mode identifications were made with a large printed version of the spectrum. The duration of the observations implies a frequency resolution of $3.75 \mu\text{Hz}$. We extended the data time series with zeros to provide an interpolated frequency spectrum that allowed us to estimate confidently the centre frequency of most spectral features to $\pm 1 \mu\text{Hz}$. Representations of the observed frequencies as power series expansions in degree did not match the precision of the observations and we resorted to least squares fits of three-node cubic spline functions to achieve good, compact representations of the frequency dependence.

Table 2 contains values of l and ν for three nodes (a, b, c) of splines, the values of the second derivatives of the splines with respect to degree at the first and last nodes (f'') and the number of points we used to derive these coefficients. Within the degree range l_a to l_c , the splines provide frequencies that match our observations to $\pm 1 \mu\text{Hz}$.

For a more detailed comparison with other observations and model predictions, the values in Table 3 can be used. These values are the frequencies of local maxima in the spectrum that we have identified with specific degree and radial order numbers and are accurate to $\pm 1 \mu\text{Hz}$ in the centre of the Table 3 degrading to $\pm 2 \mu\text{Hz}$ at frequency extremes.

Our results show no statistically significant (< 0.5 s.d.), systematic differences when compared with most previous measurements^{4,5,8}. But we do find that our frequencies average $1.25 \pm 0.23 \mu\text{Hz}$ lower than measurements from the South Pole⁶. The source of this discrepancy is unknown. Figure 3 shows that our results are consistently higher in frequency than a recent model¹². The discrepancy is greatest at intermediate degree and low radial order. Evidently there is a substantial difference between the model and the Sun below the convection zone since the high-degree frequencies in Fig. 3 that sample the upper levels agree relatively well.

There are several noteworthy features in the spectrum. In the 2–4 mHz range, we noticed that the individual modes appeared with a frequency width consistent with the duration of the observations. A frequency autocorrelation of the spectrum around 3.2 mHz confirmed this impression and further showed no systematic dependence on degree. In addition, we have fit and subtracted single sine functions of time to individual modes with very little residue. Thus we confirm previous conclusions^{4–6} that low-degree modes are coherent for at least 3 days and extend that conclusion at least to degree 70.

We found mode identification to be easiest at low frequencies and low degree. At high degree greater difficulty was probably due to confusion with temporal sidelobes and positional jitter in our observations and to frequency crowding of different modes. At low degree, we do not understand why the high frequency modes are hard to identify. The answer may be related to the increased frequency width at high frequency reported by Grec *et al.*⁶. In regard to these observation it may be worth noting that the outer boundary of the wave cavity for high frequency oscillations is located at a higher layer of the atmosphere. Here the quality of the wave reflection might decline owing to spatial inhomogeneities, radiative and other forms of leakage of the wave energy.

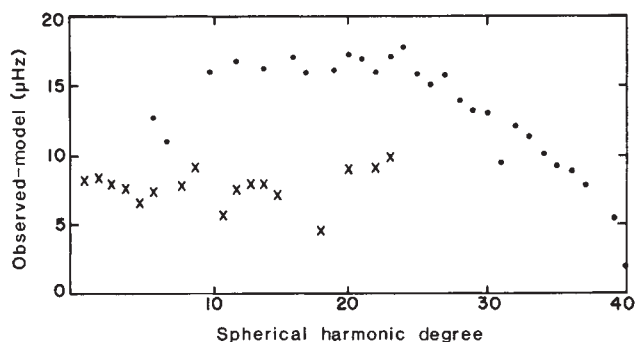


Fig. 3 Comparison of our measured frequencies with model predictions by Ulrich and Rhodes¹². ●, Radial order 12; ×, radial order 19. Radial orders between 12 and 19 shows discrepancies that fall between those illustrated. For degree values not included in the table in ref. 12, least-squares, cubic-spline interpolations provided values that appear to be accurate to well below 1 μHz .

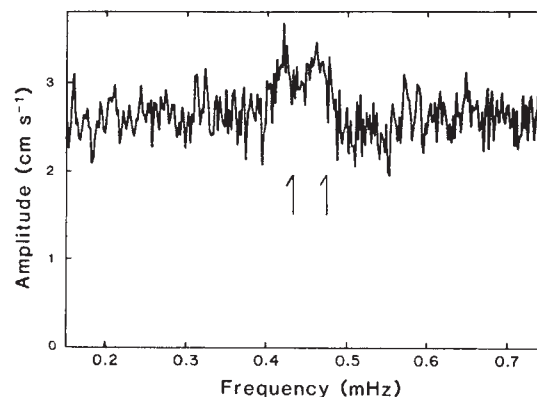


Fig. 4 Average spectrum between degrees 85 and 125. The double-peaked feature with an averaged amplitude about 6 mm s^{-1} above the background, at $\sim 450 \mu\text{Hz}$, may be related to gravity waves trapped inside the Sun. The two arrows are the upper limiting buoyancy frequencies of two cavities that trap gravity waves in a model of the Sun¹². A smooth rise of the background noise at lower frequencies was eliminated. The ordinate scale shows the average amplitude of the spherical harmonic coefficients (equation (4)) in the l range 85–125.

Our *echelle* diagrams show modes of even and odd angular order that are detected with the response of Table 1. These diagrams suggest systematic variations of strength with angular order larger than a uniform excitation leads us to expect. Further investigation is needed to verify our suspicion that selection rules may govern the strength of modes of different angular order.

Low-frequency oscillations

Between 0.6 and 1.7 mHz the spectrum shows only a few isolated features that may be unusually strongly excited modes of low radial order. There are too few of these isolated features to identify them with confidence. Below $\sim 600 \mu\text{Hz}$ the average level of the spectrum rises because of increasing instrumental, atmospheric and possibly solar noise. We looked for the oscillations reported by Hill *et al.*¹⁴, but found nothing significantly stronger than the noise at the reported sidereal frequencies. Between 410 and $480 \mu\text{Hz}$ and degrees 85–125 there is a signal of average amplitude $\sim 6 \text{ mm s}^{-1}$ per l and ν spectral bin shown in Fig. 4 that may have a solar origin. According to a typical model of the solar interior¹⁵ there is a deep-lying cavity in which gravity waves may be trapped. At degrees $\gg 1$, the frequencies of the trapped waves approach a limiting value given by the maximum buoyancy frequency that defines the cavity¹⁶. Models typically show two local maxima of the buoyancy frequency that might be expected to produce two different limiting frequencies. In the model used by Ulrich and Rhodes¹², these frequencies are 433 and $475 \mu\text{Hz}$. These values are remarkably close to the frequencies of the two peaks shown in Fig. 4. However, there is a great difficulty in understanding how high-degree gravity waves trapped deep inside the Sun may penetrate to the surface with an observable amplitude¹⁷

and this makes us cautious about a solar interpretation of the feature in Fig. 4. One possible explanation¹⁸ is that the feature is caused by low-degree modes modulating the pattern of supergranulation. The scale of the observed feature is at the upper end of the size range of supergranules but our spatial response declines at high degree so this apparent discrepancy may be an observational artefact. Although we have been unable to identify a non-solar cause, we feel that independent evidence is required before this feature can constrain models of the Sun. If it should prove to be solar in origin, its explanation will be a challenge to our understanding of stellar interiors.

We also investigated the low-frequency, low-degree region of the spectrum for evidence of g-mode oscillations spaced at equal intervals of oscillation period. There was a hint of degree 1 and 2 oscillations with average amplitudes of $\sim 1 \text{ cm s}^{-1}$ but noise that is harmonically related to a period of one day is a serious problem in our observations and more work is needed. This noise also interfered with an effort to detect the 160.0095-min period oscillation. Since the observations of this oscillation¹⁹ show no rotational splitting, it is perhaps natural to presume that its angular order is zero. The spatial symmetry of observations used to detect the oscillation leads us to also presume that its degree is even. With these presumptions, the upper limit of the amplitude of the 160-min oscillation in our observations is $\sim 18 \text{ cm s}^{-1}$ at degrees 2, 4 and 6.

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A mechanism to explain the generation of earthquake lights

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Explanations of how earthquake lights might arise have failed to show how large charge densities can be concentrated and sustained in a conductive Earth. A physical model is proposed, based on frictional heating of the fault, that solves this and related problems.

EARTHQUAKE LIGHTS (EQLs) have long been an unexplained problem in seismology. Early on, the very existence of EQL was questioned by the scientific community since no really 'testable' data existed and observations were invariably made by untrained observers. A transformation in these attitudes occurred when photographs of luminous phenomena were taken during the Matsuhiro earthquake swarm in Japan (Fig. 1), between 1965 and 1967^{1,2}. The reluctance to accept the proposal that large-scale electromagnetic disturbances are associated with earthquakes has been supported by the lack of any satisfactory theory to explain how large charge concentrations can be generated and maintained in a highly conductive Earth. However, the continuing reports of EQL³⁻⁷, especially the Matsuhiro pictures, have led to a general acknowledgement that EQLs do occur. Numerous mechanisms for generating EQL have been investigated. Because many crustal rocks are quartz rich, piezoelectric effects have received much attention⁸, although this mechanism has generally been discounted because of both the short decay time constant of the piezoelectric process⁹ and indications that piezoelectric charges may be self-cancelling¹⁰. Mizutani *et al.*¹¹ have discussed the possible role of streaming potentials produced by movement of groundwater. However, the problem remains of demonstrating a mechanism that provides for the concentration and maintenance of large charge densities near the Earth's surface. The charge relaxation time for electrostatic processes is ϵ/σ where σ is conductivity and ϵ is permittivity (typically, $\epsilon = 0.5-1.0 \times 10^{-10} \text{ F m}^{-1}$ for crustal rocks)¹². For a relaxation time of 1 s, a conductivity below $10^{-10} \text{ S m}^{-1}$ is required and is not normally found in the Earth's crust. The following theory is proposed as a solution to this dilemma.

Note that EQL phenomena do not accompany all earthquakes, indeed it seems to be the exception rather than the rule. A significant observation has been made by seismologists in the People's Republic of China. In a recent compilation of world-wide reports of EQL¹³, most occurrences were for earthquakes of magnitude 7 or greater, with only a few in the range $M = 6$ to 7 and none below $M = 5$. This provides an important test constraint for our theory.

Friction-vaporization theory

We suggest that during a large earthquake significant frictional heating of the shear zone will occur. In the proper conditions, this will lead to vaporization of water in and near the shear zone and a dramatic decrease in electrical conductivity, σ , for saturated or partially saturated rock from about 10^{-1} to $<10^{-10} \text{ S m}^{-1}$. Together with lowered conductivity, the intense shearing and the vaporization of porewater produces significant charge separation in the shear zone. Continued frictional heating produces increasing σ in the shear zone (10^{-5} S m^{-1} at 500°C ; 10^{-4} S m^{-1} at 650°C) resulting in a central conductor perhaps a few centimetres wide on the fault axis surrounded by a low σ sheath of rock containing vaporized porewater. This

central conductor will collect charge in the shear zone, and because it is hundreds of metres deep and only centimetres wide, it will concentrate the charge along its edges, where the curvature is highest. If the conductor is shallow enough, the charge concentrated along its top edge will produce an intense electric field at the Earth's surface, enhanced by the normal atmospheric potential gradient, that will then be strong enough to induce coronal discharge in the atmosphere above the fault.

Heating of fault

Various aspects of frictional heating of faults during earthquakes have recently received much attention¹⁴⁻¹⁸. Although the efficiency of frictional heating during earthquakes and the vertical distribution of stress on active faults are topics of much controversy, Sibson *et al.*¹⁹ have shown through identification of pseudotachylytes that significant frictional melting does occur on some faults. Measurements on laboratory samples²⁰ show that $>90\%$ of the energy released in stick-slip is used in frictional heating of the fault. So we can assume that, at least for some earthquakes, significant heating of the fault zone will occur.

Conductivity of rock

The study of the electrical properties of rock in a variety of geological conditions is still in its early stages, but many useful data already exist. Olhoeft^{21,22} has measured resistivity (equal to σ^{-1}) as a function of temperature for granite, basalt and hornblende schist. At 100°C , σ for vacuum dry granite is $10^{-12} \text{ S m}^{-1}$, that is >10 orders of magnitude lower than wet granite. (Similar results were found for the other rock types.) Although it may seem unexpected, as water-saturated granite is heated above 100°C at 1 atm, conductivity will approach $10^{-12} \text{ S m}^{-1}$ even though water vapour is still present in the pores.

Water vapour is often thought to be a good electrical conductor. For example, it is well known that in humid weather, conductivity of insulators will significantly increase. However, this apparent increase in conductivity is due to surface conduction in water adsorbed onto the surface of the insulator and not to volume conduction in the vapour phase. If the adsorbed water is removed through heating or evacuation, surface conduction will be significantly reduced. Steam and water vapour are in fact very good insulators with $\sigma < 10^{-14} \text{ S m}^{-1}$ (ref. 23). Rust and Moore²⁴ have measured electrical relaxation times more than 6 times greater at the base of thunderclouds than in clear air, indicating that clouds have a conductivity of the order of $10^{-15} \text{ S m}^{-1}$ (ref. 25). In fact, this is why clouds can sustain very large charge separation. Thus, for most partially saturated rocks, surface conduction has the primary role in determining conductivity. As surface water is removed, conductivity gradually decreases until only a few monolayers remain. Olhoeft²³ has found that a rock's conductivity approaches that



Fig. 1 Photograph taken 16 s after beginning of EQL at Matsushiro, Japan at 23:48 (JST), 4 December 1965 (32 mm lens, F1.9). Reproduced courtesy of J. Derr⁵.

of vacuum dry rock even when some adsorbed water remains. The first monolayers of water (including the outer Helmholtz plane, OHP) are tightly bound to crystal surfaces²⁶, so that even though some surface conduction occurs in this region, it is much less than the conduction that occurs in the more mobile layers beyond the OHP. Thus, for wet granite with pore pressure below the critical pressure of 22 MPa and at temperatures sufficient to vaporize the water, conductivity will be within one order of magnitude of the vacuum dry conductivity²³. Data for granite²² are reproduced in Fig. 2 together with a curve of estimated conductivity at 1 atmosphere pore pressure.

Charge source

Numerous mechanisms have been suggested as potential sources of charge for EQLs. Piezoelectricity and streaming potential have already been mentioned here. Due to the intense shearing inherent in earthquakes, triboelectric processes must also occur²⁷. However, the primary source of charge in our model is the vaporization of porewater during the intense shearing and heating of the fault zone. It has long been known that mechanical disruption of water droplets can produce large charges^{28,29}. The charging of water droplets by splashing or spraying was termed 'waterfall electrification' by Lenard³⁰ or by others, 'Lenard splashing'. It has also been referred to as 'balloelectrification'³¹ and 'spray electrification'³². These terms all refer to the disruption of liquid surfaces and can generate hundreds or thousands of volts. For example, explosions on supertankers at sea have occurred while being washed down with a high-power waterjet. Pierce³³ suggested the electrical charging that was developed through Lenard splashing during the washing operations was probably the cause of the discharge that ignited oil vapour and resulted in these disasters. Although this manner of separating charge is a well documented experimental phenomenon, the mechanism by which the charge is produced is not well understood. It is thought to be related to the sudden disruption of the surface layer in non-equilibrium conditions. When a new surface is created a liquid will spontaneously form a structured boundary layer whose chemical and electrical gradients differ from those of the interior of the fluid. The equilibrium distribution of ions, as well as the rate at which the boundary layer forms, will depend on the types and concentration of ions in solution. If a segment of the boundary layer is suddenly removed (by violent boiling, for example) the net charge of the resulting droplet can be very different from that of the host. Blanchard³⁴ has, in fact, found that the mechanical tearing apart of water during violent boiling

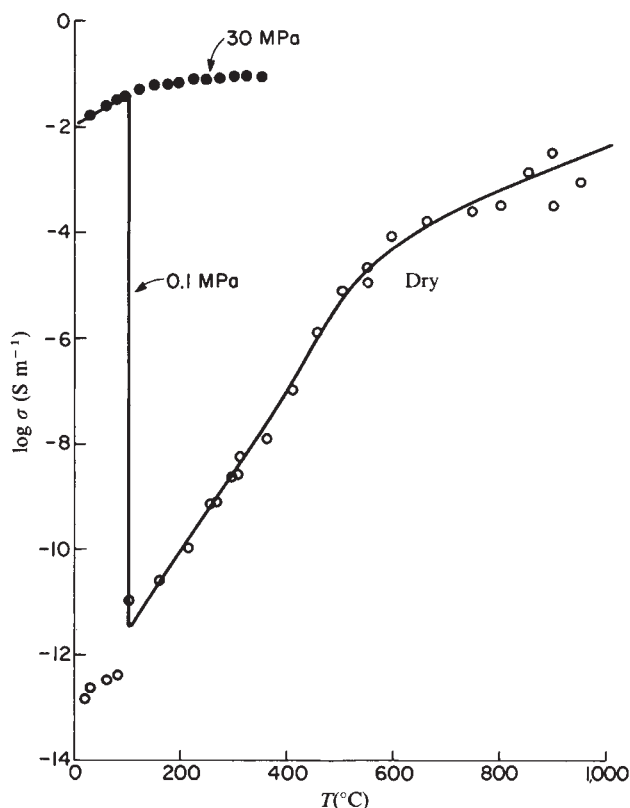


Fig. 2 Log conductivity against temperature for granite at 0.1 MPa pore pressure (data taken from ref. 22). The abrupt drop in σ at the boiling point should occur for pressures below the critical pressure (22 MPa). Open symbols are vacuum dry; closed symbols are water-saturated rock at 30 MPa.

generates charges up to 10^{-7} C g⁻¹ of evaporated water. As an example below will show, this process can provide a charge sufficient for the requirements of our model.

In applying our theory, many plausible geometries can be used that would produce circumstances favourable for EQL or other electromagnetic phenomena. For a vertical fault plane in which $\frac{\partial T}{\partial z} \ll \frac{\partial T}{\partial x}$ (where x is perpendicular to the fault and z increases with depth), temperature $T(x, t)$ satisfies¹⁷

$$\frac{\partial T}{\partial t} = \frac{A}{\rho c} + \alpha \frac{\partial^2 T}{\partial x^2} \quad (1)$$

where A is the heat source, ρc the volumetric specific heat and α the thermal diffusivity. The temperature $T(0, t)$ on the axis of a uniformly sheared fault during slip is given by¹⁷

$$T(0, t) = \frac{\tau v}{2\rho c a} \left[1 - 4i^2 \operatorname{erfc} \frac{a}{a_T} \right] \quad (2)$$

where

$$i^n \operatorname{erfc} x = \int_x^\infty i^{n-1} \operatorname{erfc} \varepsilon d\varepsilon \quad (2a)$$

$$i^0 \operatorname{erfc} x = \operatorname{erfc} x = 1 - \operatorname{erf} x \quad (2b)$$

and where τ = shear stress, v = average sliding velocity, a = fault half width and $a_T(t) = \sqrt{4\alpha t}$ is the conduction half width. Following the earthquake (duration time = t^*), we calculate temperatures in one of two ways. For $a/a_T^* < 1$, the source is diffusion dominated and we approximate it by an instantaneous release of heat per unit area = $\tau v t^*$ at $x = 0$, $t = t^*/2$. The temperature distribution is given by³⁵

$$T(x, t') = \frac{\tau v t^*}{\sqrt{\pi \rho c a_T}} e^{-(x/a_T')^2} \quad (3)$$

where $t' = t - t^*/2$ and $a_T' = \sqrt{4\alpha t'}$. For $a/a_T^* > 1$, conduction of heat out of the shear zone is not important during the earthquake. In this case, the source is approximated by a region of width $2a$ and of uniform temperature at time $t^*/2$. The solution is given by

$$T(x, t') = \frac{\tau v t^*}{4\rho c a} \left[\operatorname{erf} \frac{a-x}{a_T'} + \operatorname{erf} \frac{a+x}{a_T'} \right] \quad (4)$$

The approximations (3) and (4) give errors $<10\%$ for $t = 2t^*$ and rapidly converge on the correct solution for $t > 2t^*$. Equation (2) gives the axial temperature for $t \leq t^*$ exactly.

Values of the parameters used in the example are

$$\alpha = 0.01 \text{ cm}^2 \text{ s}^{-1} \quad (5a)$$

$$\rho c = 0.65 \text{ cal cm}^{-3} \text{ }^\circ\text{C}^{-1} \quad (5b)$$

$$v = 200 \text{ cm s}^{-1} \quad (5c)$$

$$\mu = 0.48 \quad (5d)$$

$$T_g = 10 + 30z \text{ }^\circ\text{C km}^{-1} \quad (5e)$$

Equations (5a), (5b), and (5d) are typical values found for crustal rocks¹⁷. The coefficient of friction μ is within the range of values measured both in the field and in the laboratory for San Andreas Fault gouge. Laboratory measurements indicate that in regions lacking clay-rich gouge, μ can be as large as 0.80. This would make near-surface frictional heating even more intense than we assume in the present model. Equation (5c) is generally assumed to represent an earthquake stress drop of 20 MPa (ref. 36). Although earthquake stress drops commonly range from 1 to 10 MPa (representing velocities between 10 and 100 cm s⁻¹, 20 MPa is a plausible value. In fact, one implication of our model is that during moderate earthquakes, EQL would be more likely to occur when stress drops are above average.

A thermal gradient of 30 °C km⁻¹ (equation (5e)) is added to all temperatures, although this has little effect on the near-surface phenomena. Also, we assume a 100 m unsaturated zone above the water table. The main effect of having such a low water table is to increase the near-surface effective pressure (= normal stress - pore pressure), and consequently increase the shear stress. By using a 100-m water table, shear stress at 100 m depth is 20% higher than if the water table were at the surface. Because other parameters can affect the near-surface heating, the precise depth of the water table is not critical. Effects due to heat of vaporization as water is flashed to steam are ignored here. For a shear zone containing 1% water, neglecting heat of vaporization results in at most an 8 °C error and for the purposes of the present example is unimportant. If water content were 10%, errors of as much as 80 °C could result and other processes (such as transport of heat by migration of water from the fault zone) may become important. To avoid these complications, we assume a porosity of only a few per cent. Complex effects resulting from ejection of steam from the fault zone are ignored. We further assume that pore pressure in the region of interest remains below 22 MPa because of undersaturation, dilatancy of the fault zone, high permeability¹⁷, or hydrofracture³⁷.

To estimate the stress distribution, the best data to draw on are probably those of McGarr *et al.*³⁸, who determined that static shear stress (τ_s) in the top 1 km of the crust parallel to the San Andreas Fault is

$$\tau_s = (0.90 \pm 0.47)(\text{MPa}) + (7.86 \pm 1.18)(\text{MPa km}^{-1})z. \quad (6)$$

The dynamic shear stress τ measured along the fault during sliding should in general be $< \tau_s$. However, depending on the composition of the fault gouge material, the coefficient of friction can be much greater than equation (5d). As a result, dynamic shear stress in other regions could be greater than would be calculated by equation (6). The amount of energy going into frictional heating rather than seismic radiation is also important. Laboratory experiments²⁰ have found frictional

heating to be $>90\%$ efficient for low normal stress events. Thus, for the sake of simplicity, using equations (5d) and (6) and a 100-m unsaturated zone, we take

$$\tau = 1.0(\text{MPa}) + 13.0(\text{MPa km}^{-1})z \quad z \leq 0.1 \text{ km} \quad (7a)$$

$$\tau = 1.5(\text{MPa}) + 8.0(\text{MPa km}^{-1})z \quad z > 0.1 \text{ km} \quad (7b)$$

In (equation (7a)), pore pressure above the water table is 0 and both effective pressure and shear stress increase faster than below the water table (equation (7b)) where pore pressure increases with depth.

The processes controlling fault width are poorly understood, so our choice of the width of the shear zone, $2a$, must be somewhat arbitrary. In a study of fault zone characteristics for faults as deep as 5 km in North America, Wallace and Morris³⁹ make several generalizations. They find that the widths of fault zones tend to be greater for faults having greater displacements. Faults are generally constituted of one or more claylike gouge zones in a matrix of sheared and foliated rock that is bordered by highly fractured rock. They also find the width of the gouge zone generally to be 1/100 to 1/300 the total displacement and the width of the highly fractured zone to be ~ 10 times that of the gouge zone. From similar observations Robertson⁴⁰ has found a strong correlation between total slip, u , and fault width. He finds that $2a/u$ averages 1/100 and can range in value from 1/10 to 1/1,000. He also observes that many of the fault zones with low $2a/u$ were shallow and (presumably) at low stress. Furthermore, studies of wear in metals (little work of this nature has been done with rocks) indicate that the rate of production of wear particles is proportional to stress⁴¹. We assume, in the absence of better data, that for a single earthquake the shear zone width on a fault will be proportional to displacement and stress. Relating shear stress to depth, we use in our example

$$2a(\text{cm}) = 2 \times 10^{-2} u(\text{cm}) z(\text{km}) \quad (8)$$

For earthquakes of $M = 6, 7$ and 8 , we use slip $u = 29, 130$, and 620 cm, respectively. $\sigma(T)$ is taken from Fig. 2 (using the data of Olhoef²²). Some key parameters in the example are listed in Table 1.

Figure 3a-d plots conductivity contours for different stages of an $M = 8$ earthquake. These were obtained by first calculating temperature from equations (2), (3) and (4) and then converting to conductivity by means of Fig. 2. The wedge shape contours in Fig. 3a, plotted at the end of the earthquake ($t^* = 3.1$ s), outline the shear zone, which in this example increases linearly with depth. Outside the shear zone, $\sigma > 10^{-2} \text{ S m}^{-1}$. On the edges of the shear zone, temperature rises

Table 1 Model parameters

	z (km)	τ (MPa)	a (cm)	$T(0, t^*)$ (°C)	$\sigma(0, t^*)$ (S m ⁻¹)
$M = 8$	0.001	10.1	0.01	738	4×10^{-4}
$u = 620$ cm	0.01	11.3	0.06	715	3×10^{-4}
$v = 200$ cm s ⁻¹	0.02	12.6	0.12	681	2×10^{-4}
$t^* = 3.1$ s	0.05	16.5	0.31	566	3×10^{-5}
	0.1	23.0	0.62	435	4×10^{-7}
	0.5	55.0	3.10	227	3×10^{-10}
$M = 7$	0.001	10.1	0.00	346	1×10^{-8}
$u = 130$ cm	0.01	11.3	0.01	362	2×10^{-8}
$v = 200$ cm s ⁻¹	0.02	12.6	0.03	375	4×10^{-8}
$t^* = 0.65$ s	0.05	16.5	0.06	396	8×10^{-8}
	0.1	23.0	0.13	389	7×10^{-8}
	0.5	55.0	0.65	227	3×10^{-10}
$M = 6$	0.001	10.1	0.00	170	4×10^{-11}
$u = 29$ cm	0.01	11.3	0.00	183	6×10^{-11}
$v = 200$ cm s ⁻¹	0.02	12.6	0.01	197	9×10^{-11}
$t^* = 0.145$ s	0.05	16.5	0.01	231	3×10^{-10}
	0.1	23.0	0.03	271	1×10^{-9}
	0.5	55.0	0.14	227	3×10^{-10}

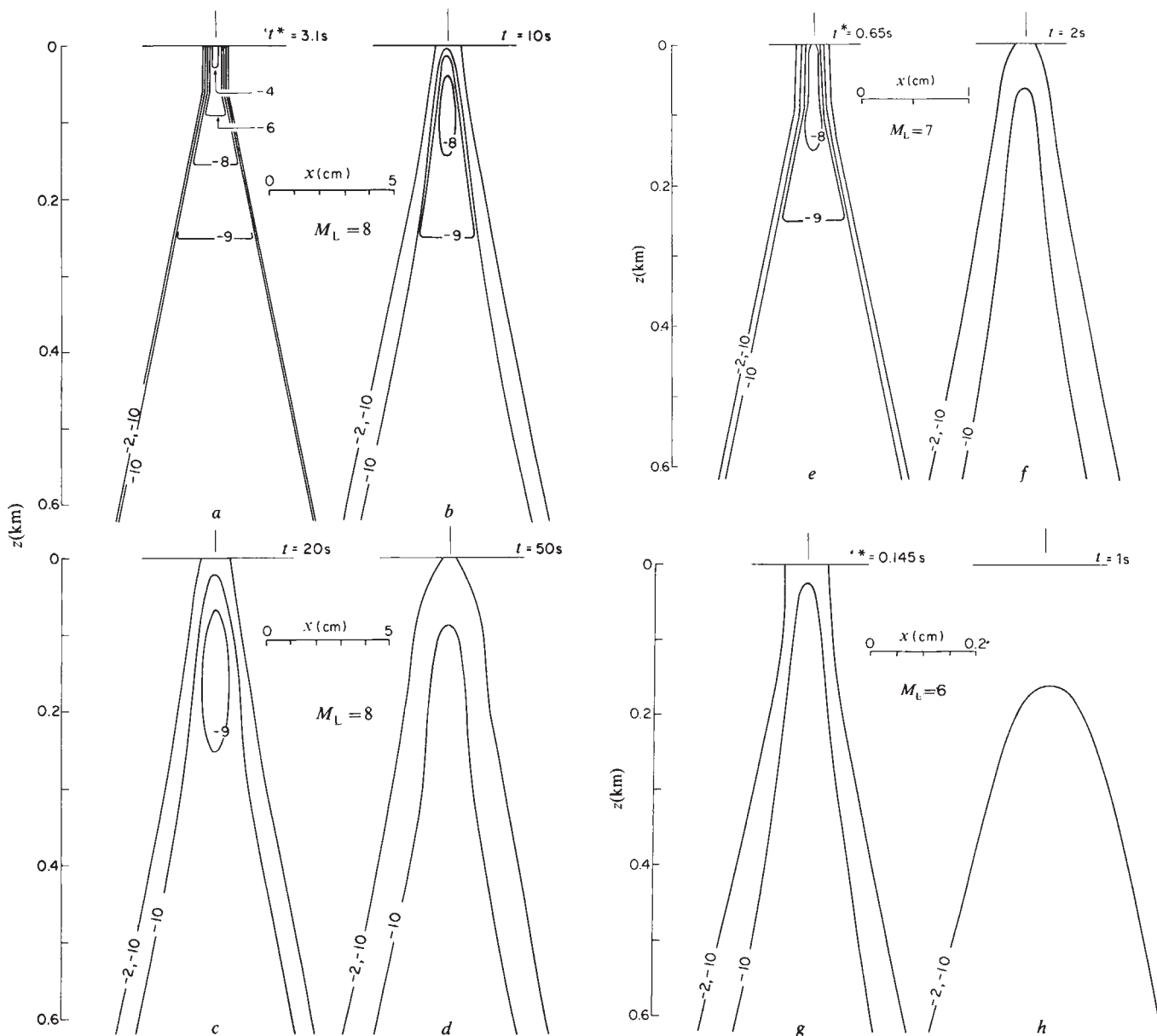


Fig. 3 Log-conductivity cross-sections (S m^{-1}) for various earthquakes $v = 200 \text{ cm s}^{-1}$, *a-d*, $M = 8$; horizontal exaggeration = 3,230:1. *e, f*, $M = 7$, horizontal exaggeration = 15,400:1. *g, h*, $M = 6$ horizontal exaggeration = 69,000:1; shear zone has not heated enough to form central conductor.

abruptly ($a_T^* = 0.4 \text{ cm}$) and $\sigma < 10^{-11}$ as water is vaporized. In the interior, temperature approaches 740°C and conductivity increases to $4 \times 10^{-4} \text{ S m}^{-1}$ near the surface, where slip is accommodated by the narrowest shear zone. The central conductor actually reaches the surface, extends to a depth of 100–200 m and averages $\sim 1 \text{ cm}$ in width. Such a narrow near-surface shear zone is not uncommon¹⁶. Below 400 m, temperature in the shear zone changes little with depth because increasing stress is balanced by increasing fault width. This leaves the conducting region bounded by an insulator $\sigma < 10^{-11} \text{ S m}^{-1}$ on the sides and $\sigma < 10^{-9.5} \text{ S m}^{-1}$ underneath. The low conductivity region in the shear zone below 400 m depends on the ratio of stress to fault width and could be decreased by 2 orders of magnitude by choosing a more complex depth dependence for these parameters. Conductivity will also vary with mineralogy. For example, conductivity of $10^{-12} \text{ S m}^{-1}$ was reported for magnetite-free dunite⁴² at 200°C . Also, increasing quartz content will tend to decrease conductivity. Thus, σ can easily vary by 2 orders of magnitude above or below the curve drawn in Fig. 2. As water vapour, charged by violent boiling and shearing, leaves the shear zone, the central conductor is

left oppositely charged. After 10 s (Fig. 3*b*), the central conductor has disappeared as the near-surface shear zone cools. However, any charge accumulated in this region will be trapped in what now has become a low conductivity zone until the water vapour can recondense. Figure 3*d* plots conductivity at 50 s, just before water vapour in the surface layer recondenses and shields any remaining charge from the atmosphere.

To estimate the charge that would accumulate near the surface, let us assume that porosity is 1% and that the vaporized water in the central conductor (Fig. 3*a*) provides 10^{-7} C g^{-1} of charge³⁴. Note that although the central conductor in Fig. 3*a* appears wedge-shaped, horizontal exaggeration is 3,230:1 and the true width-to-depth ratio is about 10^{-4} . Thus most of the charge will concentrate along the high-curvature edges of the conductor and will be further concentrated near the surface by the natural geopotential gradient. If all of this charge were concentrated in a 1-cm wide surface zone, the charge density would be $2 \times 10^{-1} \text{ C m}^{-2}$, much greater than the $5 \times 10^{-5} \text{ C m}^{-2}$ required to cause coronal discharge in air over a flat plate. Even if the efficiency of this process were as low as 10^{-3} , there would still be sufficient charge to cause EQL. In addition, any surface

irregularities would further concentrate the charge, and so would enhance this effect.

Figure 3e, f plots conductivity for a $M = 7$ earthquake. As in the previous example, the insulating layer reaches the surface. However, because of the decrease in total slip, the near-surface zone does not get as hot as it did in the $M = 8$ example. Consequently, the central conductor only begins to form and surface charge concentration will be much less than in the previous example. Also, the charge concentration will be shielded from the atmosphere after only 2 s (Fig. 3f). Figure 3g, h plots conductivity for a $M = 6$ earthquake. In this example, no central conductor develops, and so any mechanism for concentrating charge from large sections of the fault is eliminated. Our model shows that a $M = 7$ earthquake is the smallest that we would expect to produce EQL in the conditions presented in these examples. This agrees with the observations, stated earlier, that EQL primarily occur during earthquakes of $M \geq 7$.

Conclusions

We have shown that with quite reasonable physical assumptions, EQL can be generated and should be expected for at least some earthquakes. Since our mechanism for producing EQL is a near-surface phenomenon even for very large earthquakes, we would not expect these effects to occur in deep subduction zone earthquakes. This is consistent with the reported observations. Furthermore, since this mechanism is a consequence of substantial energy release during the failure process, EQL are not expected to precede the main shock. In some cases, EQL have been reported to occur moments before the main shock. These observations might be explained in the following manner. A person standing, for example, 60 km from the hypocentre of a large earthquake would first observe either EQL in the distance or perhaps the reflection of EQL off of overlying clouds. However, given a crustal shear wave velocity of $\sim 3 \text{ km s}^{-1}$, he would not feel the earthquake until 20 s later, thereby concluding incorrectly that the lights preceded the earthquake.

It is clear from the examples shown that for EQL to be generated, sufficient heat must be available to vaporize water and to form a central conductor and the pore pressure must remain low. At 10 MPa, for example, the boiling point of water is raised to 311°C , at which point $\sigma = 0.6 \times 10^{-8} \text{ S m}^{-1}$ (Fig. 2). The electrical conductivity will exceed this value at all other temperatures and an insulating sheath will not be formed. Low pore pressure at depth will occur only if the rock has no pore water or if appreciable dilatancy accompanies the earthquake. Measurements of faults³⁹ reveal that gouge zones are generally surrounded by regions of highly fractured rock ~ 10 times wider than the gouge zone. Because this fractured zone increases with increasing fault displacement, it is probably produced during the slip episodes. Consequently, co-seismic dilatancy, low pore pressure, and therefore electromagnetic phenomena may occur hundreds of metres below the surface. Development of the central conductor is strongly dependent on the near-surface shear zone width, stress displacement, and sliding velocity (equation (2)). In the example in Fig. 3a–d, the central conductor would still form for $v = 10 \text{ cm s}^{-1}$. However, for an $M = 7$

earthquake, the shear zone would no longer heat up enough to form a continuous conductor. Although there is sufficient slip in a $M = 8$ earthquake to make EQL possible over a wide range of slip velocities, the smaller slip associated with $M = 6$ to 7 earthquakes reduces the likelihood of EQL unless near-surface heating is enhanced. Large stress-drop earthquakes would have higher slip velocities and would therefore be more likely to generate EQL. Earthquakes resulting from the creation of new faults would generally fall in this class. In fact, the 1975 Haicheng⁶ earthquake, for which lights were reported, ruptured at right angles to the trend of mapped faults in the region suggesting that it may have formed a new fault. Near-surface shear stress can be increased in several ways. Gouge zones that are deficient in clay may have coefficients of friction of 0.8 or more. In such regions, shear strength could increase rapidly with depth. Regions with surface shear stress exceeding 1 MPa (ref. 38) will also have greatly enhanced near-surface heating.

EQL would not be expected to occur with all large shallow earthquakes. However, for all large shallow earthquakes we would expect transient changes in both pressure and electrical conductivity in the region around the fault zone. Secondary effects associated with this process might include rapid changes in groundwater levels and the emission of water vapour and other charged gases (CO_2 , H_2 , He, and so on) from the region around the fault zone. Increases in near-surface humidity and ground fog at the time of the Haicheng earthquake^{6,43} and indications of lights over the ocean⁴⁴ may be related to this. Interestingly, large electric fields would also occur with both the emission of water vapour¹⁵ and of gas²⁹ from the fault zone area. These fields could also result in ground glow⁴⁶ as gases are forced up through the permeable zones around, and perhaps in, the fault zone.

Sources of rapid crustal heating other than fault failure should also result in aspects of the electrical process that has been described here. Possible examples are dyke injection and magma intrusion. While the physical details are less clear, the main feature of the model presented here (the formation of an insulating sheath around the injected dyke) together with the secondary features (water-level changes, gas emission, and so on) resulting from the pressure pulse, should both occur. Dyke injection and magma intrusion may have occurred during the Matsushiro earthquake swarm⁴⁷. Because the EQL at the Matsushiro swarm are the only known ones not associated with large scale surface rupture or large magnitude earthquakes, these EQL are not easily explained by the fault heating process. An explanation in terms of dyke injection in this old volcanic zone would seem preferable in this case. On the other hand, both the 1975 Haicheng⁶ and the 1975 Hawaii earthquakes⁷ may be good examples of failure-generated EQL. The lights in the latter case were apparently viewed parallel to strike along the coast in a south-west direction.

We conclude that, even if EQL do not occur during a given earthquake, short-term transient electric and magnetic disturbances would result from both the generation and movement of charge and the short-term modification of the conductivity structure around the fault zone. These may provide useful tests of our theory.

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Complete nucleotide sequences of the T24 human bladder carcinoma oncogene and its normal homologue

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DNA sequence analysis of the activated oncogene from the T24 human bladder carcinoma line and two alleles of its normal cellular progenitor (c-Ha-ras-1) indicates that the genes encompass at least four exons, and that only a single point mutation residing within the first exon distinguishes the coding region of both alleles of the normal gene from their activated counterpart. Both versions of the gene encode a protein which is predicted to differ from the corresponding viral gene product at three amino acid residues, one of which was previously shown to represent the major site of phosphorylation of the viral polypeptide.

DNA TRANSFECTION experiments have revealed a class of dominant cellular transforming genes present in human and animal neoplasms which are capable of inducing the morphological transformation of NIH 3T3 fibroblasts^{1–8}. These neoplasms include tumours of spontaneous origin as well as those induced chemically and virally. The transforming genes detected by transfection of tumour DNA have been analysed for their sensitivity to cleavage with different restriction endonucleases. These characterizations for the most part indicate that specific transforming genes are activated in the same differentiated cell type, while different transforming genes are activated in distinct types of differentiated cells⁹. Hybridization analysis of the transfecting donor sequences which segregate with the malignant phenotype has directly implicated specific DNA sequences in this process, and has revealed a provocative relationship between cellular transforming genes and the oncogenes of acute transforming retroviruses. The transforming genes detected in human bladder and lung carcinoma DNAs were shown to be activated cellular homologues of, respectively, the *ras* genes of Harvey (v-Ha-*ras*) and Kirsten (v-Ki-*ras*) sarcoma viruses^{10–12}, whose protein product (p21) is responsible for the malignant transformation induced by these viruses^{13–15}.

The isolation of molecular clones of both the activated transforming gene from a human bladder carcinoma^{16–18} and its normal progenitor^{12,19} (termed c-Ha-*ras*-1) has led to the striking finding that the mutation which activates the proto-oncogene represents a single nucleotide substitution^{20,21}. In an effort to elucidate the structural relationship between c-Ha-*ras* and v-Ha-*ras*, as well as to explore in more depth the extent of alterations associated with the activation of the T24 human bladder carcinoma oncogene, we have isolated clones of both the activated and normal versions of c-Ha-*ras*-1 and determined the complete nucleotide sequence of each gene. To delineate the boundaries of the transforming region further, we have created vectors in which a 4.8-kilobase (kb) restriction fragment from each clone, beginning 25 nucleotides upstream from the presumed translational initiation codon of p21, was placed under the transcriptional control of the SV40 early promoter. Following transfection of these plasmids on to the Rat-1 fibroblast line, we determined that only the gene product derived from T24 cells was capable of the morphological transformation

of these cells. These results, combined with an analysis of the nucleotide sequence: (1) indicate that a single nucleotide substitution distinguishes the activated gene from its normal counterpart within the sequences encoding p21, although other sequence differences are observed in intronic and flanking regions; (2) verify the presumed translational initiation codon of c-Ha-*ras*; (3) predict that the products of these genes are closely homologous to the viral Ha-*ras* gene product, distinguished from the latter by three amino acid substitutions; (4) demonstrate that the cellular gene is composed of at least four exons; and (5) suggest that rat c-Ha-*ras* apparently engendered v-Ha-*ras* through a process of transduction involving RNA splicing and perhaps additional mechanisms.

Molecular cloning of T24 oncogene and normal c-Ha-*ras*-1 homologue

Molecular analysis of human genomic DNA with a v-Ha-*ras* probe has revealed a family of human cellular genes with homology to the Harvey murine sarcoma virus oncogene^{19,22}. Two of these homologues are detectable by high stringency Southern hybridizations; the c-Ha-*ras*-1 and c-Ha-*ras*-2 genes occur on *Bam*HI fragments of 6.6 and 3.0 kb, respectively¹². We have identified several additional homologous sequences by screening a human genomic DNA library at low stringency (D.C. and D.G., unpublished results). Recently it has been demonstrated that the c-Ha-*ras*-1 gene corresponds to the normal progenitor of the T24 and EJ bladder carcinoma transforming genes^{10–12}. To isolate this proto-oncogene, a genomic DNA library was constructed using *Bam*HI-digested lymphocyte DNA from a normal individual enriched for 5–8 kb *Bam*HI fragments by sucrose gradient fractionation. Previous Southern hybridization analysis of this DNA had shown that the two c-Ha-*ras*-1 alleles present in this individual were readily distinguished by a size polymorphism, occurring on *Bam*HI fragments of 6.6 and 7.0 kb (data not shown), as previously reported for other cell lines¹⁶. The resulting DNA fragments were cloned into the *Bam*HI site of the phage vector Charon 30A and screened with the v-Ha-*ras* probe²³. Both normal c-Ha-*ras*-1 alleles were represented among six independently arising clones analysed.

[illegible]

pT24-10 and pPBL116-4, respectively. pT24-10 was digested with *Bam*HI fragment carrying the T24 oncogene into three fragments (1.9, 2.8 kb) and sequenced using the shotgun approach described previously^{51,52}. The 1.9-kb fragment by partial DNaase I digestion in the presence of Mn²⁺, and using T4 DNA polymerase⁵³ and cloned into the *Sma*I site of phage vector (less phenotype) were used as templates in sequencing reactions in the presence of dNTPs to prime DNA synthesis by *E. coli* DNA polymerase I (large fragmentary data and remaining gaps were closed by sequencing cDNA (a gift from Dr J. Messing). Two non-transforming c-Ha-ras-1 clones containing the known sequence of the T24 oncogene a set of overlapping restriction fragments (approx. 100 bp). These DNA fragments, which spanned the region from the *Bam*HI site to the *Hind*III site of the M13 vectors for sequencing using the dideoxy method. The fragments were segmented into known exons and introns. The coding portions of the fragments are boxed. Boxed areas mark differences between the oncogene and its normal counterpart. Position 680 is not present in c-Ha-ras-1 and the first coding exon codes for glycine. Underlined nucleotides represent allelic differences in the normal allele only). The sequence AGTAAA is overlined and the sequence ATGTTGGAG is underlined. The sequence was sequenced as cloned cDNA from COS cells (provided by Y. Gluzman,

(as judged by the presence of unrelated human *Bam*HI fragments in the original phage clones) isolated in this non-selective fashion exhibited transforming activity on NIH 3T3 cells, suggesting that only the transforming allele is present in the T24 cell line.

The DNA sequence of the 6.6-kb *Bam*HI fragment containing the T24 oncogene was determined by the dideoxy chain termi-

nation method²⁴ and is presented in Fig. 1. Previous studies have shown that the transforming activity of the T24 gene is contained entirely within the 4.6-kb *XhoI-SphI* portion of the 6.6-kb *BamHI* fragment¹². To understand the structural changes underlying the phenotypic difference between the T24 oncogene and proto-oncogene, we determined the nucleotide sequence for this portion of the latter gene. Comparison of these sequences with that recently determined for the *ras* oncogene of the Harvey murine sarcoma virus²⁵ reveals an open reading frame of 567 base pairs (bp), interrupted by three intervening sequences, with the capacity to encode a protein of molecular weight (*M_r*) 21,300 with striking homology to the viral p21. As summarized in Fig. 1, the two human genes differ by three single base changes in addition to a 6-bp deletion in the far 5' region of the normal c-Ha-*ras*-1 gene. One of these changes, which alters the 12th amino acid residue of the proto-oncogene from a glycine to a valine in the T24 oncogene, was recently shown to be responsible for the activation of the oncogene^{20,21}. The other two changes, which occur within the first and last intervening sequences, were not present in a second nontransforming allele (see below).

Although the v-Ha-*ras* and human c-Ha-*ras*-1 coding sequences are only 88% homologous, most of the nucleotide differences occur at the third position of codons or are otherwise neutral; the resulting human cellular p21 proteins are identical to the viral protein at all but 3 out of 189 amino acid residues (Fig. 2). One of these differences is at the aforementioned amino acid 12, where an arginine residue is found in the viral p21. Another noteworthy difference between the viral and cellular peptides occurs at position 59; an alanine residue is encoded by the human cellular genes in place of the threonine which is thought to be the site of autophosphorylation catalysed by the viral p21 (ref. 25). Given the close similarity of the v-Ha-*ras* and T24 transforming gene products, this observation suggests that phosphorylation at Thr 59 may not be essential to viral transforming activity²⁶.

Further comparison of the v-Ha-*ras* and c-Ha-*ras*-1 sequences reveals significant nucleotide homology in the 5' region (Fig. 2). There are two short stretches of homology immediately upstream of the translation start codon, and another highly conserved region of ~120 bp found about 1 kb farther upstream in the human genes. The DNA separating these regions of homology may well represent an intron in the 5' region of the human genes. This notion is consistent with the good potential donor and acceptor splice sites²⁷ present at the ends of this sequence. Thus, the lack of sequences in the viral oncogene homologous to this 1-kb stretch of human DNA may be the result of the splicing of the presumptive intervening sequence during retroviral transduction of the corresponding rat cellular homologue. Alternatively, a 15-bp direct repeat sequence flanking the 1 kb of DNA in the human genes (see Fig. 2) could be implicated in the deletion of this DNA region during transduction by the involvement of a mechanism reminiscent of transposon excision.

Sequences which may constitute eukaryotic transcription initiation signals are found in the region 5' to the p21 coding segments of the T24 oncogene. A potential Goldberg-Hogness box^{28,29}, which is thought to determine the specificity of transcription initiation by RNA polymerase II (ref. 30), is present at position 1,334 (TCTAATT) and precedes by 29 nucleotides the sequence, TCAGCCC, which is similar to that observed for RNA cap sites³¹. In addition, 83 nucleotides 5' to this potential cap site is found the sequence GGTAACCT (position 1,280) which resembles the consensus sequence GG^CTCAATCT (ref. 32) that appears 70–80 bp upstream of the RNA start of many eukaryotic promoters. If transcription of the c-Ha-*ras*-1 genes begins in this region and extends to the polyadenylation site at position 3,751 (see below), the resulting spliced transcript would have a predicted size of 1.25 kb. Taking into account the addition of polyadenylate residues, the size estimated for the T24 mRNA is somewhat higher than the 1.1–1.2 kb

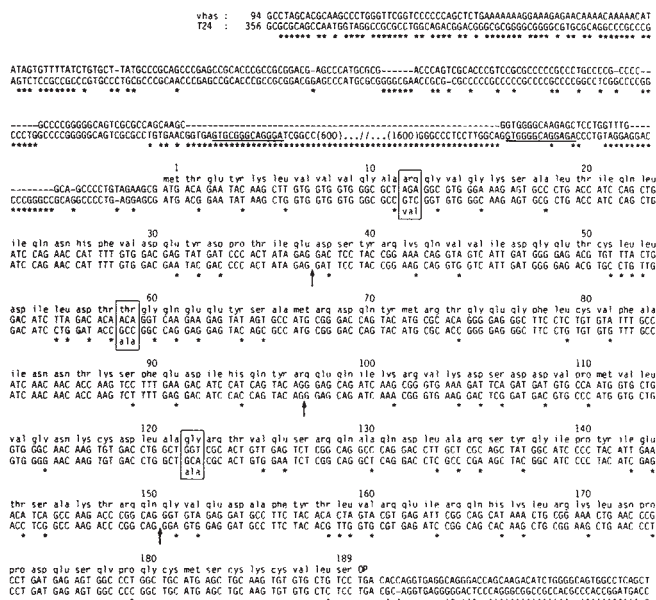


Fig. 2 Sequence comparison of the oncogenes from Harvey murine sarcoma virus and the T24 human bladder carcinoma cell line. The nucleotide sequence for v-Ha-*ras* is from Dhar *et al.*²⁵. Asterisks mark differences in the two sequences. Areas where such differences lead to amino acid changes are boxed. Arrows show the positions of introns in the T24 oncogene. Extensive homology exists between the 5'-noncoding region from v-Ha-*ras* and an area of the T24 gene ~1,200 nucleotides upstream of the ATG initiation codon, suggesting the presence of an intron in the 5'-noncoding region of the T24 oncogene. A direct repeat in the 5'-noncoding region of the T24 oncogene is underlined.

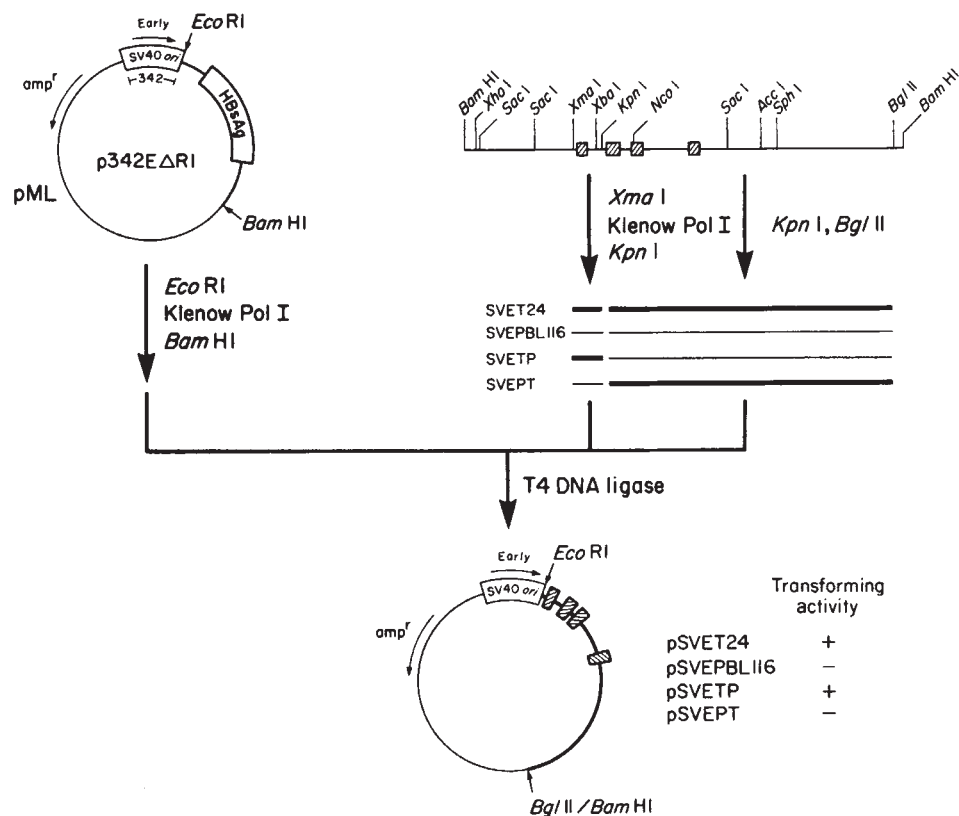
observed by Northern analysis^{11,12,16}. Direct analysis of the RNA synthesized from the T24 oncogene will be necessary to establish firmly the actual site of transcription initiation and the presence or absence of a 5'-untranslated intervening sequence.

Immediately 3' of the *SphI* site (nucleotide 4,776 of Fig. 1), in the region not essential for transforming activity, is a stretch of repeated DNA 800–900 bp in length, consisting of the 28-bp consensus sequence, CACTCCCCCTTCTCTCCAGGGGACGCCA. This repeat is reiterated 29 times in the plasmid subclone of the T24 oncogene used for sequencing. However, subsequent restriction mapping revealed an apparent deletion of 120–150 bp in this region suffered during propagation in *Escherichia coli*. Thus, the overall length of the *BamHI* fragment containing the oncogene is close to the 6.6 kb determined by agarose gel electrophoresis. Interestingly, the 0.4-kb difference in *BamHI* fragments noted above for the two normal c-Ha-*ras*-1 alleles maps between the *SphI* and *ClaI* sites (data not shown), indicating that the previously noted size polymorphisms for the T24 oncogene¹⁶ probably represents fluctuations in the number of reiterations of this repeat unit.

Expression of T24 oncogene and normal c-Ha-*ras*-1 gene under SV40 early promoter control

To ascertain whether expression of the T24 coding elements identified above was sufficient for transforming activity, a chimaeric plasmid was constructed in which the prelude region of the T24 oncogene was replaced with a promoter element derived from SV40 which directs early viral transcription. An equivalent plasmid was constructed from the normal c-Ha-*ras*-1 gene and was also examined for transforming activity. The structure of each chimaeric plasmid is shown in Fig. 3. A 342-bp fragment³³ spanning the promoters for SV40 early and late viral RNA synthesis was oriented so that transcription beginning within the early promoter would continue into the T24 oncogene or normal c-Ha-*ras*-1 gene at the *XmaI* site 25 bp

Fig. 3 Construction of chimaeric SV40 early promoter-human c-Ha-ras-1 plasmid derivatives. The plasmid p342EARI, in which the expression of hepatitis virus surface antigen is directed by the SV40 early promoter, was derived from p342E (ref. 33) by removal of the upstream *EcoRI* site. p342EARI was successively digested with *EcoRI*, extended to flush ends with *E. coli* DNA polymerase I Klenow fragment and digested with *Bam*HI; the resulting large vector fragment was ligated in a three-component reaction with two fragments derived from either the T24 oncogene plasmid subclone, pT24-10, or the normal c-Ha-ras-1 gene plasmid subclone, pPBL116-4, to reassemble the protein coding regions of these genes behind the viral promoter element. These fragments were prepared by: (1) digesting pT24-10 or pPBL116-4 with *Xma*I, repairing the ends with Klenow fragment, digesting with *Kpn*I and isolating the resulting 330-bp blunt *Kpn*I fragment containing the first exon coding sequences, and (2) digesting each plasmid with *Kpn*I and *Bgl*II and isolating the 4.5-kb *Kpn*I-*Bgl*II fragments containing the remaining exons. The resulting chimaeric plasmids, pSVET24 and pSVEPBL116-4, join the SV40



early promoter to sequences beginning 25 bp upstream of the coding regions of the T24 oncogene and proto-oncogene, respectively. Two further derivatives were made by exchanging one of the two fragments used in each construction, yielding genes of a hybrid nature, one portion being derived from the T24 oncogene, the other from the normal gene. The resulting reciprocal hybrids, pSVETP and pSVPT, contain the first exon coding sequences of the T24 and normal c-Ha-ras-1 genes, respectively. Transformation assays were performed by transfecting $0.5\text{--}1.0 \times 10^6$ Rat-1 cells (60 mm dishes) with plasmid DNA by the calcium phosphate precipitation method⁵⁶ in the presence of 5 μg of rat carrier DNA. Following a 16-h exposure to the DNA precipitate, cells were treated with 20% glycerol in phosphate-buffered saline for 45 s, and the media then replaced. After 24 h, cells were split to 100-mm culture dishes. The medium was changed every 3–4 days, and foci were scored after 14–21 days.

upstream of the p21 initiator methionine. On transfection of these constructs into normal Rat-1 fibroblasts, only the chimaeric plasmid containing T24 sequences, pSVET24, exhibited transforming activity. The efficiency of transformation was comparable with that observed with the natural T24 oncogene ($\sim 1.2 \times 10^3$ foci per μg) and resulted in foci with similar morphology (data not shown). Thus, substitution of the SV40 early promoter for the sequences 5' to the p21 coding elements of the T24 oncogene does not alter transforming activity.

Since the difference in transforming ability between the T24 oncogene and normal c-Ha-ras-1 genes on transfection did not seem related to the level of transcription of these genes, molecular hybrids similar to those described by Tabin *et al.*²⁰ were constructed from the chimaeric plasmids to confirm the location of the region containing the functional alteration. Specifically, the 330 bp *Xma*I-*Kpn*I fragment extending from the viral promoter juncture to the middle of the first intron of the c-Ha-ras-1 genes was exchanged (Fig. 3). Figure 3 gives the results obtained with the two reciprocal hybrids obtained in this fashion, pSVETP and pSVEPT. Only the hybrid containing the 330-bp fragment derived from the T24 oncogene exhibited transforming activity ($\sim 0.8 \times 10^3$ foci per μ g), thus confirming analogous experiments with the complete natural genes^{20,21} localizing the functional change to the region encompassing the first coding exon of the gene. Because two nucleotide differences between the T24 and normal c-Ha-ras-1 gene occurred within this segment of DNA, it was possible that either an amino acid alteration or an intron change, perhaps resulting in aberrant RNA processing, was responsible for the activation of transforming activity. To resolve this ambiguity, the corresponding DNA fragment from the second non-transforming allele isolated from the normal lymphocyte DNA library was

subjected to sequence analysis. It was found that the second allele of c-Ha-ras-1 does not show a nucleotide change relative to the oncogene in the first intron, but carries several changes in the 5'-untranslated region (see Fig. 1) and a silent change in the codon for His 27. Thus, the only consistent difference between the T24 oncogene and its nontransforming alleles is the G→T transversion affecting amino acid 12.

Several characteristics of the transcription directed by the T24 oncogene were inferred by analysing the nucleotide sequence of T24 oncogene-specific cDNA cloned from total poly(A) RNA synthesized on transfection of the COS-7 cell line³⁴ with the plasmid pSVET24 (Fig. 3). Although RNA synthesis originates from the SV40 early promoter in this chimaeric plasmid rather than the natural site of transcription initiation in the T24 oncogene, sequences predicted to lie within introns by analogy with the *v-Ha-ras* gene were spliced correctly and are not present in the cDNA. Furthermore, this RNA was polyadenylated 16 bp following the sequence AGTAA which occurs ~400 bp downstream of the p21 termination codon (Fig. 1). The same polyadenylation signal has been found in the long terminal repeat (LTR) of the mouse mammary tumour virus³⁵ and is very similar to the sequence AATAAA found 10–20 bp upstream of the site of poly(A) addition in most eukaryotic mRNAs³⁶.

Discussion

The nucleotide sequences determined for the T24 oncogene and normal c-Ha-ras-1 gene indicate that each encodes a p21 highly homologous to the product of the v-Ha-ras viral oncogene. The observed homology provides strong support for the idea that the coding region of the Harvey murine sarcoma virus transforming gene was derived from a spliced version of the rat cellular sequences homologous to the human c-Ha-ras-1

gene^{19,37}. The human and viral p21s differ by only three amino acid residues despite more extensive nucleotide divergence in the coding region, implying a strict evolutionary constraint on the structure of these gene products. In addition, the substantial nucleotide conservation observed between the far 5' region of the human cellular genes and sequences proximal to the viral p21 translational start suggests an important regulatory function for this region.

We have demonstrated that sequences upstream from the T24 oncogene initiation codon may be functionally replaced by a viral promoter element derived from SV40, indicating that this region does not encode additional translatable sequences required for transforming activity. An analogous plasmid derived from the normal c-Ha-ras-1 gene does not exhibit transforming activity. We have also studied the transforming ability of hybrids derived from these chimaeric SV40-c-Ha-ras-1 plasmids in which the first encoding exon of each gene was exchanged. These results have confirmed the finding of others that the ability of the T24 oncogene to transform normal cultured rodent fibroblasts may be attributed to a structural alteration in the protein encoded by this gene^{20,21}. The lack of transforming activity in the SV40 early promoter-normal c-Ha-ras-1 chimaeric plasmid further argues against the notion that the T24 oncogene is able to transform due to increased expression relative to the normal c-Ha-ras-1 gene. However, these results do not exclude the possibility that the product of the normal gene can be expressed at levels sufficient to induce a malignant phenotype in certain conditions. In fact, it has been reported that the same proto-oncogene can be activated by the LTR of the Harvey sarcoma virus²³. It is conceivable that the differences between our results and those of Chang *et al.*²³ are a consequence of the relative levels of transcription supported by the SV40 early promoter versus the Harvey MuSV LTR in the cell lines studied (Rat-1 versus NIH 3T3, respectively). Furthermore, the amounts of 3'- and 5'-untranslated c-Ha-ras-1 sequence present in these two experiments are quite different; Chang *et al.*²³ used a 2.9-kb *SacI* fragment which extends from 500 bp upstream of the initiation codon to about 200 bp downstream of the polyadenylation site.

The observation that the phenotype associated with expression of the T24 and viral *ras* oncogenes is dominant and that replacement of glycine at position 12 constitutes the primary site for activation of the transforming ability of these proteins suggests that the biochemical function of the oncogenic p21 proteins is altered rather than defective relative to that of the corresponding normal human and rat cellular proteins. In principle, however, amino acid substitutions at position 12 could

result in a protein with a transdominant, negative phenotype, which has been found for mutations in proteins active as higher-order multimers, such as the lactose repressor^{38,39}. The availability of the human c-Ha-ras-1 gene sequence should facilitate *in vitro* mutagenesis to define further this aspect of p21 function.

The molecular mechanism by which p21 transforms normal cells into the malignant state is unknown. Two unusual properties have been associated with the polypeptide specified by the v-Ha-ras oncogene: the protein displays a strong affinity for GTP and GDP^{40,41}, and can apparently phosphorylate itself in an intramolecular reaction at a threonine residue^{25,40-42}. The p21 proteins encoded by v-Ha-ras and c-Ha-ras-1 share many properties: both proteins are of similar size, both are synthesized as a precursor protein which becomes associated with the plasma membrane; and both display guanine nucleotide-binding activity⁴². In other respects, however, the proteins differ. For example, whereas much of the v-Ha-ras protein is phosphorylated on a threonine residue, only a minute fraction of the c-Ha-ras protein is phosphorylated, and that on a serine residue⁴². In addition, no autophosphorylation of the cellular p21 protein is observed. In view of the recent observation that the viral protein is phosphorylated *in vivo* at a threonine residue 59 amino acids from the initiator methionine²⁶, our results provide a rationale for these differences. One of the three amino acid differences which distinguish the v-Ha-ras from c-Ha-ras-1 proteins involves a change at the amino acid 59. Whereas a threonine residue occurs at this position in v-Ha-ras²⁵, an alanine residue is present in both the normal and activated c-Ha-ras-1 (Fig. 1). At present, the significance of this phosphorylation event, both *in vivo* and *in vitro*, is unclear, but bears some resemblance to the observation that elimination of the major phosphorylated tyrosine residue in pp60^{src} (the transforming protein specified by Rous sarcoma virus) by site-directed mutagenesis has no observable effect on the function of the protein⁴³.

Analysis of the differences in p21 function manifested by the activation of the T24 transforming gene may lead to a better understanding of the manifold aspects of cellular growth altered during the process of malignant transformation. Ultimately, the high level synthesis in heterologous hosts (for example, bacteria) of the gene products specified by this oncogene and its normal counterpart should make available sufficient material to initiate such biochemical studies.

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Transcription of DNA injected into *Xenopus* oocytes is influenced by template topology

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We have assayed the transcription of the herpes simplex virus thymidine kinase gene on circular and linear plasmids after injection into Xenopus oocytes. The circular plasmids appear to be transcribed at least 500 times more efficiently than the linear plasmids. Moreover, when the circular templates are allowed to form functioning transcription complexes, and are then linearized in situ, the synthesis of accurate transcripts is substantially reduced.

THE possibility that the topological state of eukaryotic DNA is important in regulating its biological activity has become attractive for several reasons. A change in the torsional stress of DNA can have radical effects on its structure. Untwisting of B-form DNA can cause local regions to adopt altered configurations such as cruciforms^{1,2}, left-handed 'Z DNA'^{3,4}, or locally melted stretches in A+T-rich sequences⁵. Altered structures such as these might act as signals in the DNA, which could be recognized by proteins and thereby serve a regulatory role.

There are several biological studies which suggest that the topological state of DNA may have a role in gene regulation. For example, K. Nasmyth (personal communication) has shown that a change in the superhelical density of a normally silent yeast mating type gene, carried on an extrachromosomal plasmid, accompanies the derepression of that gene in a mutant background. Furthermore, structures exhibiting the properties of non-B-form DNA have been inferred to occur in native chromatin from their susceptibility to attack by single-strand specific nucleases⁶. These nuclease-sensitive sites are of particular interest as they tend to be associated with putative regulatory sequences of active genes. The possibility that intra-strand stem loops may form *in vivo* has been suggested by the nucleotide sequences of spontaneous mutants of polyoma virus capable of growth on F9 teratocarcinoma cells. In two independently isolated mutants extra nucleotides were found inserted near the viral origin of replication in a way that would extend the base-paired stem of a possible intrastrand hairpin loop⁷. Finally, evidence for the existence of Z DNA *in vivo* has been suggested from immunofluorescence studies of *Drosophila* polytene chromosomes using antibodies specific for Z-form DNA⁸.

The alternative structures adopted by DNA *in vitro* at physiological ionic concentrations can be induced by negative supercoiling in circular DNA molecules. The question of whether eukaryotic chromosomal DNA exists under torsional stress is an open one. As chromosomal DNA is thought to be constrained in domains, it could, in principle, be placed under localized torsional stress^{9,10}. However, no eukaryotic enzyme analogous to the prokaryotic gyrase, which actively untwists DNA, has yet been discovered¹¹. Although histone-depleted chromosomal DNA is supercoiled, most of the supercoiling can be accounted for by the presence of nucleosomes which package the DNA *in vivo*¹². In experiments designed to test the possibility that chromosomes *in vivo* may be under torsional stress, the cross-linking reagent psoralen has been used¹³, which has a higher affinity for underwound than relaxed DNA. The amount of psoralen bound to DNA was measured before and after nicking the DNA *in vivo* with gamma rays. Although this technique readily detected negative superhelicity in prokaryotic DNA, the bulk of eukaryotic DNA was found to be relaxed. However, transient or local unwinding might not have been detected in these studies and could therefore be present in some fraction of the chromatin without being observed.

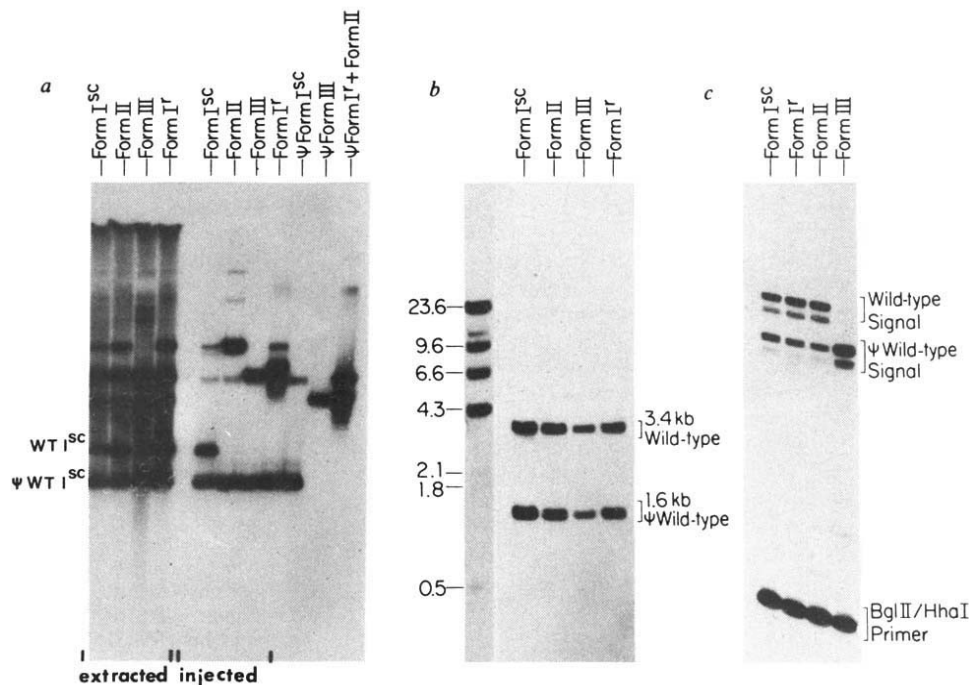
We have attempted to examine the possibility that torsional stress is required for gene expression by studying the transcription of circular and linear genes in amphibian oocytes. A covalently closed molecule cannot dissipate torsional stress unless a DNA strand is broken. A linear molecule, however, unless attached to nuclear structures, will dissipate torsional stress by rotation of the free ends. Earlier experiments, in which the expression of genes carried on circular and linear templates was compared, have shown that linear molecules are transcribed less efficiently than circles (reviewed in ref. 14). It has been suggested that the template may have to be equivalent to a supercoiled domain of the chromosome to be transcribed¹⁵, but other explanations, such as the degradation of linear molecules by exonucleolytic attack¹⁶, could not be ruled out. However, in the results reported here we not only demonstrate a much more dramatic effect of template circularity on transcriptional efficiencies than has been reported previously, but also rule out the possibility that this effect is an artefact due to the degradation of linear DNA.

Circular, but not linear, DNA is transcribed after injection into *Xenopus* oocytes

To assess the transcriptional efficiency of linear DNA molecules it is not only necessary to know the amount of RNA produced, but also how much of the template DNA survives in the oocyte. In these experiments we have used the normal and a modified herpes simplex virus (HSV) thymidine kinase (tk) gene, so that relative stability of the different topological forms of DNA, as well as their rates of transcription, could be measured in the same cell. The modified tk gene has a 10-base pair (bp) deletion in the DNA encoding the 5' untranslated sequences of tk mRNA. This small deletion does not affect the expression of the tk gene, but it does allow its transcript to be readily distinguished from the wild type tk transcript by a primer extension assay¹⁷. The 10-bp deletion renders the product of primer extension on the transcript of the modified gene 10 bases shorter than that of the wild type gene, and therefore distinguishable by gel electrophoresis. The plasmid carrying the modified gene, which we refer to here as the pseudo-wild type gene, was also designed to be distinguishable from wild type plasmid, on the basis of both the size of the plasmid template and the locations of various restriction enzyme sites. Thus, the fates of the wild type and pseudo-wild type templates as well as their transcripts can be compared after co-injection into the same oocytes.

Wyllie *et al*¹⁶ have previously shown that circular double-standard (ds) DNA is conserved in the oocyte nucleus and assembled into chromatin. In confirmation of these findings Fig. 1 shows that supercoiled covalently closed circles (form I^{sc}), relaxed covalently closed circles (form I') and nicked circles (form II) of the wild-type tk gene are all conserved to the same extent as the pseudo-wild type gene that was co-injected as supercoiled DNA. In all cases a considerable fraction of the

Fig. 1 The fate of different forms of DNA injected into oocytes, and assays of their transcription efficiency. **a**, Analysis of DNA conformations as injected into oocytes, and after incubation of injected oocytes for 24 h. An autoradiograph of DNA electrophoresed through a 1% agarose gel, first in the absence of ethidium bromide, and then in the presence of $2 \mu\text{g ml}^{-1}$ of the dye. This facilitates the separation of nicked circles (form II) from relaxed covalently closed circles (form I'). The DNA was nicked by depurination, denatured, transferred to nitrocellulose and detected²⁸ using a radiolabelled *Bgl*II-*Sma*I internal fragment of the tk gene²⁹. The mixtures of DNA that were injected are shown in the middle of the panel, and the markers for the various forms of pseudo-wild type (ψ WT) DNA are shown at the extreme right. DNA extracted from injected oocytes is shown on the left. The form of wild type gene injected is denoted at the top of each gel slot. Supercoiled (form I^{sc})



pseudo-wild type plasmid was co-injected as an internal control in each case. The two plasmids, wild type and pseudo-wild type, are of different sizes as the tk DNA fragment that is inserted in the wild type plasmid is larger (3.4 kb) than the insert in the pseudo-wild type plasmid (2.1 kb). The main point to emerge from this panel is that the various circular forms of the wild type gene all become covalently closed and supercoiled. In contrast, the linear molecule (form III) generated by a restriction enzyme cut in the vector does not lead to the formation of covalently closed supercoiled DNA. In this particular gel it is hard to assess the survival of linear molecules because other forms of DNA co-migrate with linears. The survival of linear tk is more clearly demonstrated in **b**. **b**, DNA extracted from injected oocytes, digested with *Bam*HI and *Hind*III, electrophoresed on a 1% agarose gel and detected by Southern blotting as described above. Digestion releases a 3.4-kb *Bam*HI fragment from the wild type gene, whereas *Bam*HI cuts the pseudo-wild type gene 20 bp downstream from the mRNA initiation site at a synthetic *Bam*HI linker site, and *Hind*III cuts at a synthetic *Hind*III linker site 1.6 kb away¹⁷. The form of wild type DNA injected is denoted at the top of each gel slot; pseudo-wild type DNA was co-injected in the supercoiled form in each case. When injected as a linear molecule (form III) and incubated for 24 h, the tk coding sequences of the wild type gene persist in a quantity similar to the pseudo-wild type coding sequences. **c**, Transcript map of RNA extracted from oocytes injected with supercoiled pseudo-wild type DNA and different topological forms of wild type DNA as denoted at the top of each gel slot. The transcript mapping methods described elsewhere¹⁹ were used, except that 10 mM EDTA was added to the oocyte lysis buffer to aid recovery of DNA. The various circular forms of wild type tk plasmid produce transcripts at about the same level, whereas linear wild type tk DNA produces no detectable transcripts.

DNA was found to be covalently closed and supercoiled. It has previously been shown that DNA is relaxed immediately after injection and that the appearance of supercoils reflects the formation of minichromosomes¹⁶. The background of nicked circles, full length linear molecules and nucleolytically digested fragments that appears as a continuous smear is attributable to the deposition of some of the DNA in the oocyte cytoplasm during injection: it is known that when DNA is injected into the oocyte cytoplasm it is known to be progressively degraded¹⁶.

In contrast to the fate of the three topological isomers of the circular plasmid, linear DNA is not detectably ligated into circles to form minichromosomes, and after 24 h shows evidence of exonucleolytic degradation. However, an appreciable amount of the injected linear DNA survives at or very near to full length. Similar observations have been made with SV40 DNA¹⁸. In our experiments with the tk gene, the linear DNA was generated by cleavage of the vector at the unique *Sal*I site, well away from the inserted tk gene, so that the intact gene sequence should still be present in much of the partially degraded DNA. To obtain a more accurate estimate of how much of the tk coding sequence remained intact, the DNA extracted from oocytes was digested with *Bam*HI and *Hind*III to excise DNA fragments carrying the gene. These were separated by gel electrophoresis, transferred to nitrocellulose and detected using as a probe a radioactively labelled DNA fragment derived from an internal portion of the tk gene. The amount of hybridization to the DNA fragment derived from the wild type tk gene that had been injected in the linear form was then compared to the amount of hybridization to the DNA

fragment derived from the pseudo-wild type gene that had been injected in the circular form into the same oocytes. Although there are some inherent limits to quantitations using this agarose gel blotting assay, we are able to derive a reasonably accurate comparison between the amounts of hybridization to molecules injected into the same oocytes either as equimolar mixes of linears and circles, or as mixes of the different topological forms of the circular plasmid. From the results of experiments like that shown in Fig. 1, we estimate that at least two thirds as many intact coding sequences of the tk gene survived in oocytes for 24 h after injection as linear molecules compared with those injected as circles.

Faithful initiation of transcription of both wild type and pseudo-wild type genes was assayed using a primer extension assay that provides a quantitative measurement of accumulated transcripts¹⁹. All of the circular forms of injected DNA gave rise to transcripts at the same level of efficiency. In contrast to the behaviour of the three circular forms of the plasmid, linear DNA does not seem to serve as a transcription template despite its persistence in substantial amounts. These transcript mapping results are shown in Fig. 1. The degradation of linear DNA is not an adequate explanation for the failure to detect proper initiation. In experiments such as those shown in Fig. 1, where linear wild type and control circular pseudo-wild type genes are co-injected, we cannot detect any specific transcripts from the linear template. The sensitivity of this assay is diminished because in heavily exposed autoradiographs the signal from circular pseudo-wild type template obscures any signal from linear wild type template (data not shown). If the linear wild

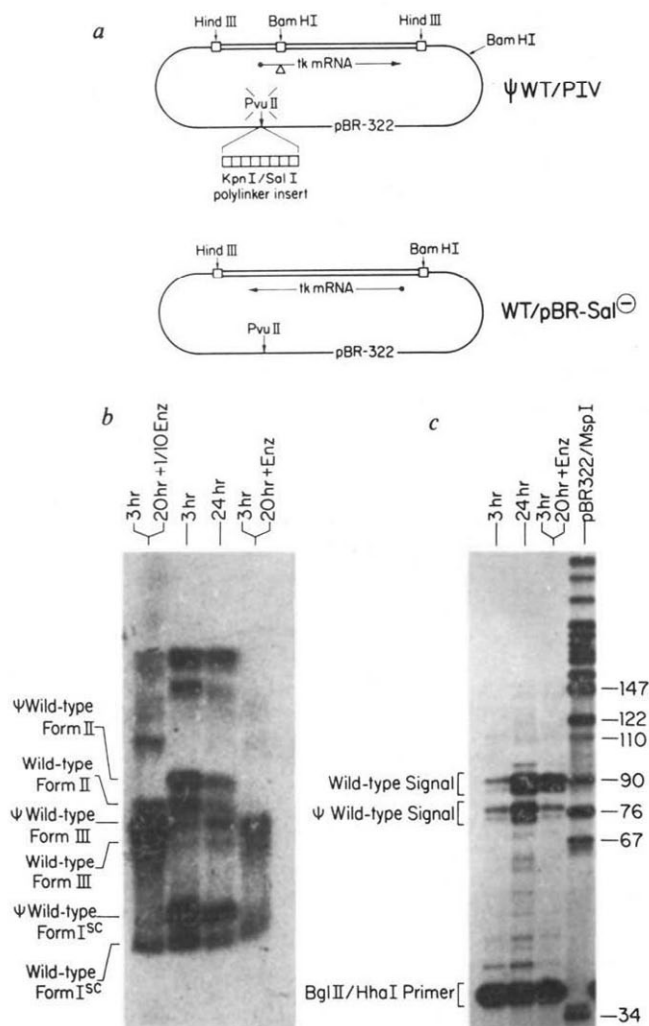


Fig. 2 The requirement of continued circularity for transcription. **a**, Two tk plasmids, which can be distinguished by size, tk transcript and susceptibility to restriction endonucleases. The wild type and pseudo-wild type plasmids described in Fig. 1 produce distinguishable transcripts, but were further modified so that one (the pseudo-wild type) could be cut by the restriction enzymes chosen, and the other (wild type) would remain uncut. The wild type gene has its *KpnI* site mutated by digestion with *KpnI*, trimming of the ends with *S₁* nuclease and re-ligation. Although this produces a frameshift in tk coding sequences it does not affect the production of tk RNA. This gene was inserted into a pBR322 vector which had had its *SalI* site mutated by similar means. This plasmid (WT pBR-Sal⁻) therefore has no *SalI* or *KpnI* sites. The enzymes *KpnI* and *SalI* were reconstituted from lyophilized stocks (BRL) at 10–20 units μl^{-1} , then dialysed in 10 mM potassium phosphate pH 7, 1 mM EDTA and 1 mM 2-mercaptoethanol. The pseudo-wild type plasmid¹⁷ was recombined with the *KpnI*-deficient plasmid, and inserted into a vector into the *PvuII* site of which multiple synthetic *KpnI* and *SalI* restriction sites had been ligated. This vector was in turn constructed from the pBR322 Sal⁻ vector so that all the *KpnI* and *SalI* sites of this gene (ψ WT/PIV) are located at a single site, well removed from the tk coding sequences. The plasmids are easily distinguishable by size because the wild type plasmid (WT/pBR Sal⁻) contains only 148 bp upstream of the mRNA initiation site, and lacks the small *BamHI*/*HindIII* fragment of pBR322. The pseudo-wild type plasmid (ψ WT/PIV) contains viral DNA sequences 480 bp upstream of the mRNA initiation site, as well as the *BamHI*/*HindIII* fragment of pBR322, resulting in a net difference of ~600 bp. **b**, An equimolar mixture of the wild type and pseudo-wild type plasmids described above was injected into oocytes. This panel shows the fate of the DNA, analysed by methods described in Fig. 1 legend, after different incubation intervals, as well as with or without restriction enzyme reinjection. After 3 h both plasmids were supercoiled, and continued to be so for 24 h. If restriction enzymes (*SalI* and *KpnI*) were injected at 3 h the pseudo-wild type DNA was cut, and the wild type DNA was left intact. This is evidenced by the fact that the supercoiled pseudo-wild type DNA was no longer observed, and was replaced by material migrating in the form of linear DNA molecules. It was found that this result could be obtained at a 10-fold reduced restriction enzyme concentration. **c**, Transcript mapping of RNA extracted from the same oocytes as analysed in **b**. Transcripts were observed from both genes after 3 h, and continued to accumulate to a higher level at 24 h. Transcripts of the linearized pseudo-wild type gene (20 h + Enz) did not seem to accumulate appreciably beyond the level of mRNA observed 3 h post-injection.

type tk gene is injected by itself the assay is much more sensitive and we do detect some accurately initiated tk transcripts (data not shown). To quantitate the detection of such low levels of RNA we carried out reconstruction experiments in which RNA from oocytes injected with circular tk genes was serially diluted with RNA from uninjected oocytes. The resulting RNA mixtures were assayed by primer extension and the transcription signal compared with that obtained from oocytes injected with linear DNA. These experiments show that the level of transcription detected from linear molecules is 500 to 1,000-fold less than that produced by circles. Even this exceptionally small amount of transcription may not be coming from linear templates. Although the DNA for injection was digested with a large excess of restriction enzyme, we cannot exclude the possibility that a very small fraction escaped digestion, nor can we rule out the possibility that a very small proportion of the linear tk genes recircularized in the oocytes. Our methods for detecting topological forms of the injected DNA are not sensitive enough to detect the formation of less than 0.2% circles. A small proportion of linear DNA is indeed ligated into material that co-migrates with linear dimers, indicating that the oocyte has some ability to ligate free DNA termini. It is therefore possible that all of the observed transcription derives from a small fraction of circular molecules and none from the linear templates.

The template must remain circular for continued efficient transcription

Linear DNA molecules may never be assembled into transcription complexes because of interference by the free ends of the molecule, rather than because the molecule is free to dissipate torsional stress in the vicinity of the gene. To test whether a circular, actively transcribing template might be deactivated if linearized, we constructed a plasmid that can be efficiently cut by restriction enzyme digestion after it has assembled into a functioning transcription template. Restriction enzymes do not digest chromatin efficiently^{20,21}, so a series of contiguous recognition sites for the restriction enzymes *SalI* and *KpnI* spanning more than 100 bp was inserted into a vector by ligating together synthetic oligonucleotide 'linker' molecules. We assumed that when this vector DNA molecule is packaged in nucleosomes, one or more of the restriction sites in the contiguous series of linkers would be exposed to restriction enzyme attack. The pseudo-wild type gene was inserted into this vector. The *SalI* site of pBR322 and the *KpnI* site of the tk gene had previously been mutated so as not to be cut. Our plan was to inject oocytes with this *SalI*-*KpnI* 'polylinker' vector carrying the pseudo-wild type gene, allow minichromosomes to form and transcription to begin, and then reinject the oocytes with concentrated *SalI* and *KpnI* restriction enzymes. In this type

of experiment it is particularly important to use an internal control as the nonspecific effects of the restriction enzymes on transcription in the oocyte could not be predicted. Thus, the wild type gene was constructed on a vector lacking recognition sites for the *SalI* and *KpnI* enzymes, and co-injected into the same oocytes as the pseudo-wild type gene carried on the polylinker vector. Reinjection of oocytes carrying the two plasmids with a concentrated mixture of *SalI* and *KpnI* was predicted to quantitatively cut the plasmid carrying the pseudo-wild type gene to the linear form, yet leave the wild type gene as a circular molecule thus serving as an internal control.

Figure 2 shows that the injected DNA of both the wild type plasmid and the pseudo-wild type polylinker plasmid was supercoiled after 3 h, and therefore assembled into chromatin. Three hours after the first injection the oocytes were injected with restriction enzymes. Following incubation for a further 24 h, all the pseudo-wild type tk plasmid was linearized, whereas the injection of restriction enzymes did not affect the topological conformation of wild type gene in the same oocytes. Transcription was measured by primer extension. Accurate tk mRNA was detectable after 3 h from both the wild type and the pseudo-wild type templates. This mRNA synthesis accumulated in 24 h from both genes if the oocytes were not reinjected with restriction enzymes. In the case where the *SalI* and *KpnI* restriction enzymes were injected 3 h after the plasmids were initially introduced into the oocytes, the ratio of transcripts from the two genes changed substantially in that the transcription signal of the pseudo-wild type gene did not increase beyond the level accumulated during the first 3 h. This implies that the linearized molecules are transcribed much less efficiently than the circular molecules despite the fact that they had originally been functioning templates.

In the experiment shown in Fig. 2 the transcription signal from the linearized pseudo-wild type tk gene was reduced fourfold compared with the wild type gene. However, we believe that this difference, derived from a pool of oocytes, may be an underestimate of the change in signal. Analysis of single oocytes (Fig. 3) showed that there were two classes of signals. Some oocytes had accumulated much larger total amounts of transcript than others, and in all these cases the ratio of product from circular wild type tk genes to linear pseudo-wild type tk genes was in excess of 10:1. The rest of the oocytes had much lower amounts of both transcripts, and the ratio of the two kinds of transcript remained equivalent despite the fact that parallel analysis of the DNA showed that the pseudo-wild type gene had been linearized in every case (data not shown). Although we do not have a definitive explanation for this second type of signal we suspect it arises from oocytes where total transcription ceased as a result of the enzyme injection. Taken as a whole, we believe these experiments show that even though a molecule may be assembled into transcriptionally active chromatin as a circle, its activity is diminished or possibly even abolished when it is linearized.

Other polymerase II genes require a circular template

A further series of experiments was conducted to test whether the loss of faithful initiation on linear DNA is a peculiar property of the HSV tk gene promoter. A good candidate for a promoter that might be expected to function efficiently as a linear template in the oocyte is the adenovirus major late (ML) promoter. Transcription from this promoter is comparatively efficient in a cell-free system for polymerase II transcription when tested as a linear DNA fragment²²; in contrast, the linear tk gene is particularly inefficient in a similar cell-free system (R. Roeder, personal communication). Specific initiation of transcription from the ML promoter was tested using an *S₁* mapping procedure²³. A separate promoter having opposite polarity, the IVa2 promoter, resides on the same DNA fragment that we used to test for major late promoter function. It is of interest to test the activity of this promoter as it lacks any apparent homology to the consensus TATA sequence, and does

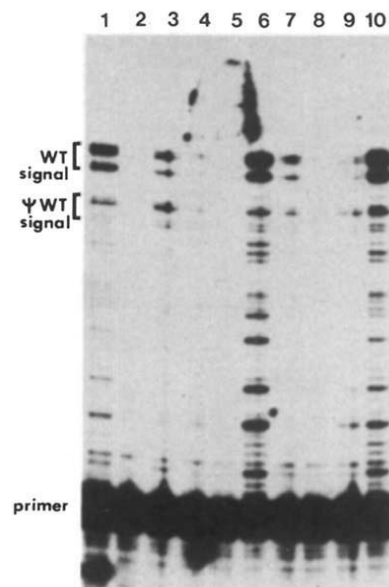


Fig. 3 Analysis of transcripts produced by individual oocytes injected with a mixture of wild type and pseudo-wild type tk genes as described in Fig. 2 legend, and reinjected with restriction enzymes after 3 h. The figure demonstrates the different classes of signal described in the text. Lanes 1, 6 and 10, oocytes having a large transcript signal, with a ratio of wild type to pseudo-wild type signal of greater than 10; lanes 3, 4, 7 and 9, oocytes with a low signal which is equal for both wild type and pseudo-wild type; lanes 2, 5 and 8 show no signal. Parallel analysis of the DNA (not shown) demonstrated that in every case the pseudo-wild type gene had been linearized.

not function in an *in vitro* polymerase II system²². A map of the relevant region of adenovirus genome, and the probes used are shown in Fig. 4b.

The adenovirus ML promoter functions in the frog oocyte to produce transcripts with the same 5' ends as those found in adenovirus-infected HeLa cell RNA (Fig. 4a, lanes 1, 5). Although the IVa2 promoter does not function in an *in vitro* system, it does function in the oocyte to produce transcripts with heterogeneous 5' ends (Fig. 4a, lane 1). Putative transcription initiation sites used by the IVa2 gene are also heterogeneous in adenovirus-infected cells²⁴, and some are similar to those observed in injected oocytes (Fig. 4a, lanes 1, 5).

When the plasmid containing the two adenovirus promoters was linearized at the *SalI* site, and then injected into oocytes, transcription from both promoters was reduced to negligible levels (Fig. 4a, lane 2). To show that transcription was occurring normally in these oocytes the HSV tk gene was co-injected together with an adenovirus gene, and the transcription of both was assayed simultaneously. In all cases the oocytes produced tk RNA with the same 5' termini as RNA extracted from HSV-infected cells (Fig. 4a, lanes 3, 4, 6). The conclusion that polymerase II promoters do not function well as linear molecules can therefore be extended to all three promoters, and most strikingly to the adenovirus ML promoter, which is known to function as a linear molecule in the RNA polymerase II cell-free systems²².

Discussion

The experiments presented here show that linear DNA molecules carrying genes transcribed by RNA polymerase II function inefficiently as transcription templates assayed after injection into oocytes. We have ruled out the possibility that degradation of linear DNA can account for the relative inefficiency of transcription from these templates. In addition, we have demonstrated a much more dramatic difference in the transcriptional efficiency of linear and circular DNA than has been reported previously¹⁴. One difference in the approach we

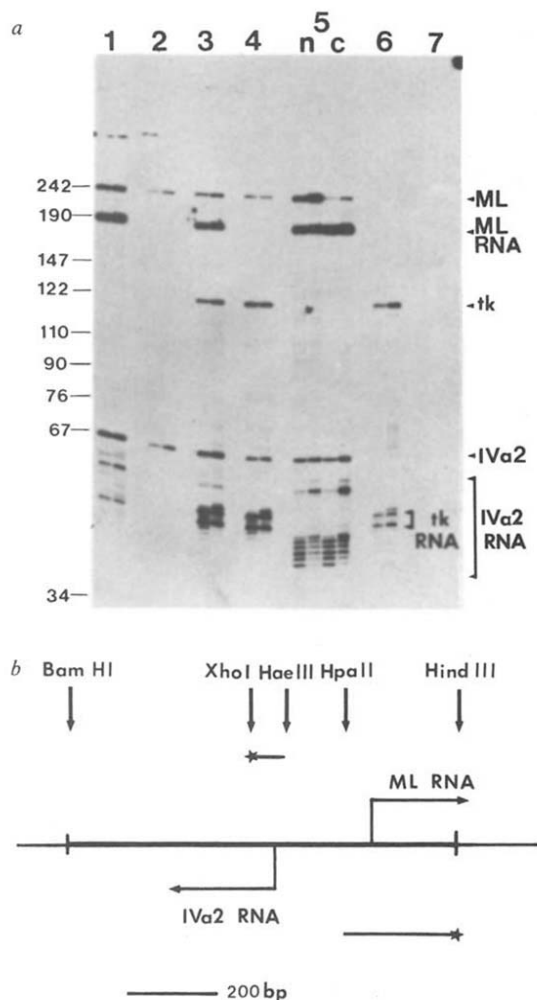


Fig. 4 *a*, Autoradiograph of a polyacrylamide gel separating the products of S_1 analysis. Each pair of lanes shows an analysis of RNA as described by McKnight *et al.*¹⁹ using two different concentrations of S_1 nuclease (2,000 units ml^{-1} and 200 units ml^{-1}) and mixtures of probes. Lanes 1–4, RNA analysed from oocytes injected with supercoiled adenovirus plasmid (lane 1); plasmid linearized at the *SalI* site (lane 2); supercoiled adenovirus plasmid co-injected with supercoiled tk plasmid (lane 3); and linearized adenovirus plasmid co-injected with tk plasmid (lane 4). Lanes 5n and 5c, analysis of RNA extracted from adenovirus-infected cell nuclei (lane 5n) or cytoplasm (lane 5c; the RNA was a gift of J. Lewis). Lane 6, RNA extracted from HSV-infected cells. Lane 7, RNA extracted from uninjected oocytes. The positions of probe DNAs are shown on the right (ML, tk, IVa2) together with the characteristic positions of probes shortened by S_1 treatment to a length diagnostic of the 5' ends of the relevant RNA molecules (ML RNA, tk RNA, IVa2 RNA). These positions are known from analysis of the RNA preparations with individual probes. *b*, A map of the regions of the adenovirus genome used to make the probes for ML and IVa2 RNA.

Methods: The probe for RNA from the major late promoter (ML RNA) was prepared by opening the plasmid at the *HindIII* site at position 6,231 on the adenovirus map³⁰, 5' labelling with ^{32}P ATP and polynucleotide kinase, secondary digestion with *HpaII* at position 5,988, and strand separation on an 8% gel¹⁹. The probe for IVa2 RNA was prepared by opening the plasmid at the *XhoI* site at position 5,777, 5' labelling, secondary digestion with *HaeIII* at position 5,848 and strand separation on an 8% gel. The probe for tk RNA was prepared as described elsewhere¹⁹ by labelling at the *BglII* site followed by secondary digestion with *EcoRI* and strand separation.

a stem-loop structure has been raised¹⁷. Formation of such a stem-loop might be favoured in a topologically constrained molecule by untwisting of the DNA^{1,2}.

A second interpretation we entertain is that circular templates may function more efficiently because they allow bound RNA polymerases to cycle around the template. It is not the case that RNA polymerase transcribes the circular template continuously to form stable RNA, because S_1 mapping experiments show that the transcripts from injected DNA have a 20–40-fold greater abundance of authentic 5' termini compared to read-through transcripts that fully protect the S_1 probe (ref. 19 and Fig. 4). However, we cannot rule out the possibility that RNA polymerases remain preferentially engaged with the circular template without transcribing it, and thereby gain greatly enhanced access to a promoter by means of unidimensional diffusion on the DNA.

As a third consideration it is possible that all transcription complexes on both circular and linear DNA are unstable relative to the interval of time in which we measure accumulated RNA. Were this the case, it could be argued that linearization of a functioning transcription template has no effect on its output of authentic transcripts. Instead, the reduction in mRNA accumulation observed following linearization could simply reflect a failure of new transcription complexes to form as a consequence of their linearized state. This hypothesis predicts that template would continue to synthesize correctly initiated RNA for a measurable time after linearization. Such a prediction may be tested by a pulse-labelling experiment, conducted at varying intervals following the linearization of a functioning template. If it is found that a transcribing template does continue to direct synthesis of its gene product following linearization, it would seem that linear molecules transcribe poorly because they are blocked in some way from initially forming a functioning complex. For example, the free ends or topologically relaxed state of a linear molecule might be incompatible with the assembly of nucleosomes, or disrupt the arrangement of nucleosomes. Unlike circular DNA injected into frog oocytes, linear molecules are not assembled into chromatin with the physiological 200-bp nucleosome repeat²⁷. If the failure of linear DNA to be properly assembled into chromatin were to account for its inefficient transcription, then our

have taken is that we have assayed the accurate formation of 5' termini on the RNA, whereas other quantitative studies have measured total RNA complementary to the injected DNA. Nonspecific initiation by RNA polymerase II, or possibly by another type of RNA polymerase, may not be so severely affected by the topological form of the template. The inefficiency of the linear RNA polymerase II template is far more marked than that of linear RNA polymerase III templates. In contrast to the 500–1,000-fold decrease in transcription of linear polymerase II genes, linear polymerase III templates (carrying 5S or tRNA genes) injected into oocytes specify the correct product and are reduced in activity by less than an order of magnitude^{14,25,26}. As well as assaying the expression of DNA injected as linear molecules we have extended our analysis to show that even though a transcription complex may be set up on circular minichromosomes, transcription from these molecules is inhibited when the template is linearized.

We cannot determine whether the difference in transcriptional efficiency of circular and linear molecules resides at the level of transcription initiation, elongation of the nascent chain, or even transcription termination. However, here we consider three general mechanisms that may account for the observations. First, it may be that each round of transcription is substantially augmented by the torsional stress that is unique to a closed circular template. For example, a transcription control sequence might act to engage RNA polymerase only when converted to a non-B-form DNA conformation, and such a conversion might require a topologically constrained template. It may be relevant to note that the transcription of the tk gene in oocytes has been shown to require at least three sequence elements upstream of the mRNA initiation site for accurate and efficient initiation of transcription¹⁷. Inverted repeat sequences are present in two of these elements, and the possibility that they may interact in

restriction enzyme reinjection experiments, which linearize fully formed minichromosomes, would suggest that a linear molecule cannot maintain the chromatin conformation necessary for its assembly into a transcription complex. Whereas our experiments do not define the level at which a circular DNA molecule is recognized as different from a linear, the surprisingly large difference in transcription we have ob-

served suggests that the topology of a DNA molecule has a fundamental role in the transcription process.

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LETTERS TO NATURE

Size of a γ -ray burster optical emitting region

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Most models of γ -ray bursters require the formation of a thermal plasma, whose properties are relatively insensitive to its mode of formation. Radiation at lower photon energies or during quiescence may be more diagnostic of the underlying cause of the γ -ray bursters. Searches for quiescent γ -ray bursters at low photon energies require an accurate position. Recently, three such accurate positions have been published¹⁻³ which have allowed X-ray³⁻⁵, optical⁶⁻⁹, IR¹⁰ and radio¹¹ searches for the GRB counterpart. We present here new limits on the quiescent optical and IR flux of the 19 November 1978 burster³. We shall use the measurement of the optical flux during outburst⁸ to place a lower limit on the size of the optical emitting region.

This measurement of the optical flux during outburst was made from a blue emulsion archival photographic plate exposed in 1928⁸. On this plate was an image that was presumably formed by optical radiation during a burst by the 19 November 1978 γ -ray burster. The small (8 by 18 arc s) error box for the 1928 image allows for very deep searches for the quiescent optical counterpart.

We observed the region around the 1928 optical error box with the MASCOT CCD camera¹² on the McGraw-Hill 1.3-m telescope on the first two nights of November 1981. The bandpass used was defined by the UV cutoff of the CCD ($\sim 3,700$ Å) and by an HA-11 filter ($\sim 6,250$ Å) which is similar to a $B + V$ magnitude system. The pixel size was 1.2 arc s which was smaller than the seeing disk due to the relatively large zenith angle for the observations. We obtained a total of 305 min of exposure. The telescope was shifted slightly between each exposure, and each frame was independently dark field subtracted and flattened. When the 21 frames were co-added, no image was visible above the 2σ confidence level in or near the 1928 optical error box. This limit corresponds to 6.7 mag fainter than star number 11 of Fishman *et al.*⁶ ($m_v = 17.13$ mag, $m_B - m_v = 1.64$

mag) in the 3,700-6,250 Å bandpass. The exact magnitude limit is uncertain due to a lack of measured comparison stars in the passband. Our procedure flattened a starless area of the sky to 0.3% (r.m.s.) of the background. (A ~ 23 mag object appears in a position which is consistent with the polarized radio source 'B' which was reported by Hjellming and Ewald¹¹.) For a reasonable upper limit on the distance of 1 kpc (ref. 13) (the burster is at high galactic latitude), the absolute magnitude of the system is fainter than roughly +15 in the bandpass used. With this limit, it is hard to see how any non-degenerate star can be in the burster system¹⁴.

M. H. Liller obtained deep B and V plates on the CTIO 4-m telescope on 1 October 1981, both of which appear to show a faint object at the same location within the 1928 optical error box⁵. These images were measured with a PDS microdensitometer; detections were at confidence levels of 3.2σ and 4.9σ above the grain noise for the B and V plates respectively. We have re-examined both of these plates after it was learned that they were exposed with a Racine wedge, which creates a secondary image for each star which is 6.8 mag fainter than the primary image. No star is positioned such that its secondary image will appear near the 1928 optical error box. The secondary images allow the magnitude sequence of Fishman *et al.*⁶ to be extended to the plate limiting magnitude. The image on the B plate has an apparent magnitude of 22.9 ± 0.3 mag, while the apparent magnitude on the V plate is 21.5 ± 0.4 mag, which would indicate a red object. If the object on Liller's plate is real, then it must be highly variable ($\Delta m \geq 2$). This variability would be strong evidence that the object is the true counterpart and not just a background star. However, when viewed by eye, these images do not inspire confidence in their reality. Hence, despite the low probability of two significant grain enhancements occurring in the same place (within 1 arc s) in a small error box, we prefer to think of the images on Liller's plates as possible detections.

On the night of 1-2 December 1981, C. Telesco and one of us (G.R.R.) examined the 19 November 1978 error region in the IR at 2.2 μ m. The InSb photometer on the Infrared Telescope Facility (IRTF) 3.0-m telescope was used. They searched to a K magnitude of 18.8 mag and found no object in the 1928 optical error box to the 3σ level of confidence. Once again, for a reasonable upper limit on the distance of 1 kpc, we find $M_K \geq 8.8$ mag; a faintness achieved by few stars¹⁵.

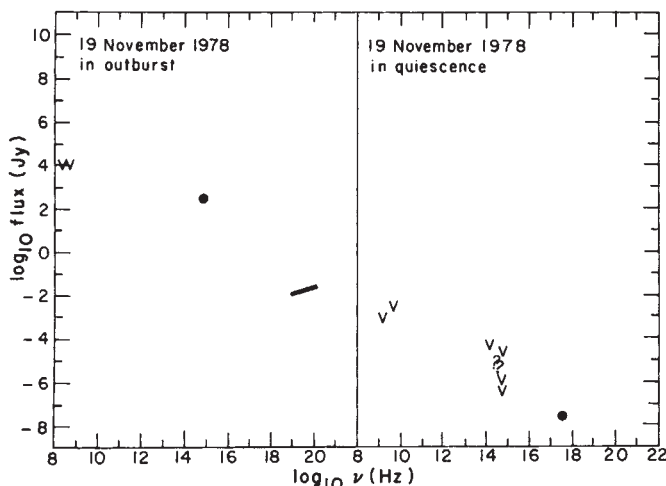


Fig. 1 Spectrum during outburst and quiescence of the 19 November 1978 γ -ray burster. 'V' indicates an upper limit, while '?' indicates a possible detection. The outburst data have been collected from refs 8, 16, 17. The data for the quiescent γ -ray burster have been collected from refs 6, 8, 9, 11, 18 and this work.

From a variety of sources^{6,8,9,11,16-18}, we have collected observations of the 19 November 1978 burster during both outburst and quiescence. For each observation, the measured flux has been plotted versus frequency in Fig. 1. Unfortunately, most of the observations are either upper limits or questionable detections.

If the γ -ray bursters have a high space density, then they would contribute significantly to the local invisible mass density. However, the γ -ray burster density cannot exceed Oort's upper limit on the local invisible mass density¹⁹. This fact can be used to limit the scale of distances to γ -ray bursters, or equivalently, to limit the average γ -ray burster luminosity;

$$\frac{Nm}{(4\pi D_0^3/3)} \leq 0.088 \frac{M_\odot}{\text{pc}^3} \quad (1)$$

The mass of an individual γ -ray burster is m . D_0 is the average distance to a γ -ray burster which is observed with a fluence of S_0 . N is the total number of γ -ray bursters inside a volume of radius D_0 . N will be greater than the total number of observed γ -ray bursters with a fluence greater than S_0 (this statement can be confirmed for any luminosity function and any reasonable distribution in space). With S_0 equalling $2 \times 10^{-4} \text{ erg cm}^{-2}$, a literature search^{17,20-24} reveals 17 bursts with a greater or equal γ -ray fluence. It is thought that the γ -ray burster phenomenon is related to neutron stars^{25,26}, so that most probably $m \geq 1.4 M_\odot$ (ref. 27). The mean γ -ray burster luminosity, L_0 , is $4\pi D_0^2 S_0$. The distance D to a γ -ray burster with observed fluence S is given by

$$D = D_0 \left(\frac{S_0}{S} \right)^{1/2} \quad (2)$$

where L is the luminosity of that γ -ray burster in units of L_0 . From equations (1) and (2) and the limits on N and m , the distance to the 19 November 1978 γ -ray burster is constrained by

$$D \geq 3.2 \text{ pc}(L)^{1/2} \quad (3)$$

Similarly, the average γ -ray burster luminosity is constrained to be $> 4 \times 10^{35} \text{ erg}$.

With the assumption that the optical radiation from the 1928 flash is the result of a thermal process, we can derive a lower limit on the size of the optical emitting region. The observed optical fluence (f) from the 1928 flash can be related to the

emittance (F) on the surface of the optical emitting region by

$$\left(\frac{D}{R} \right)^2 \frac{f}{\Delta\lambda} = F \quad (4)$$

In equation (4), $\Delta\lambda$ is the bandpass ($9.8 \times 10^{-6} \text{ cm}$) and R is the radius of the optical emitting region. The optical flux f is observed⁸ to be $(4.2 \times 10^{-7} \text{ erg cm}^{-2})/t$, where t is the duration of the optical flash. We do not know the value of F ; however, we know that it cannot exceed the Rayleigh-Jeans emittance provided that the optical light is being emitted by a thermal process;

$$F \leq \frac{c_1}{c_2} \frac{T}{\lambda^4} \quad (5)$$

λ is $4.4 \times 10^{-5} \text{ cm}$ and c_1/c_2 equals $2.60 \times 10^{-5} \text{ erg cm s}^{-1} \text{ K}^{-1}$. T is the temperature of the optical emitting region, which must be equal to or less than the temperature of the γ -ray emitting region. For the 1978 burst, Teegarden and Cline²⁸ found a γ -ray temperature of 500 keV ($5.8 \times 10^9 \text{ K}$). The equality sign in equation (5) is valid when the optical light comes from a hot, optically thick region. With the limit on T , equation (5) becomes

$$F \leq 4.0 \times 10^{22} \text{ erg s}^{-1} \text{ cm}^{-3} \quad (6)$$

From equations (3), (4) and (6), we can place a lower limit on R of

$$R \geq 100 \text{ km}(L/t)^{1/2} \quad (7)$$

Equation (7) is based on the conservative distance limit from equation (3). If instead, a reasonable¹³ distance of 100 pc is adopted, then the radius of the optical emitting region must be $> 3,000 \text{ km t}^{-1/2}$.

In a physical situation which may be similar to γ -ray bursters, the duration of optical flashes from X-ray bursters nearly equals the X-ray duration²⁹. This leads us to believe that the γ -ray burster optical and γ -ray durations are similar³⁰ (the 1978 burst had a FWHM duration of 3 s in γ -ray energies). By further making the assumption that $L \sim 1$, we deduce that the size of the optical emitting region is more than an order of magnitude larger than that of a neutron star. This is a conservative lower limit, because for the equality in equation (7) to hold, the entire local invisible mass must be composed of γ -ray bursters and kT of the optical emitting region must be $\sim 500 \text{ keV}$. Another way of stating this result is that the γ -ray emitting region is evidently too small to emit the observed optical flux. This suggests that the optical flux comes from elsewhere in the system, perhaps from an accretion disk or a companion star. Any such system component must satisfy the severe optical and IR limits on the absolute magnitude which were reported above. The presence of an accretion disk or a companion star would suggest that the energy source for the 19 November 1978 burster is not inside the neutron star.

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First detection of radio emission from a dwarf nova

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The dwarf novae represent a class of cataclysmic variable stars that typically exhibit random optical outbursts of 2–6 mag with mean outburst periods of 10–150 days. Dwarf novae are close binary systems composed of a late-type star which fills its critical Roche lobe and a white dwarf companion. The white dwarf is surrounded by a luminous accretion disk sustained by mass transfer from the late-type star. The disk is the seat of the eruptions. Although radiation has been detected from dwarf novae from IR through X-ray energies, radio emission has never been reported from these objects. We describe here a search for radio emission at 4.75 GHz from dwarf novae that has been carried out with the 100-m telescope at Effelsberg, West Germany. We have searched for radio emission from six dwarf novae and a source was discovered at the position of SU UMa. The source could only be detected during optical outbursts and was below the threshold during quiescence. We suggest here that the radio emission arises from a non-thermal process.

SU UMa was observed with a double horn receiver system. System parameters and times of observation are listed in Table 1. The observations consist of scans in elevation, 15 arc min long, that are centred on the optical position of SU UMa, with the offset horn situated 8 arc min east. In fair weather conditions, effects of clouds are mostly cancelled out with the double horn system, but the scans suffer from source confusion by neighbouring sources. At the galactic latitude of SU UMa ($+30^\circ$) most confusion sources can be expected to be of extragalactic origin. Source counts¹ near 5 GHz suggest a probability of $16 \pm 9\%$ for finding a source stronger than 1 mJy in the Effelsberg beam. To smooth the effects of source confusion we scanned the optical position with different parallactic angles. Due to the sidereal time periods available for observation, the directions of the scans varied by $\pm 35^\circ$ with respect to the celestial meridian. After rejecting poor scans obtained in unacceptable weather, 123 scans of SU UMa were found to be suitable for further analysis.

Each scan consists of thirty-one 3-s integrations separated by 30 arc s. A linear baseline was subtracted from all scans by using a mean of eleven data points at both ends of the scans. This subtraction would be expected to fail if strong confusion sources exist; however, inspections of the individual scans do not show such sources around SU UMa. The scans were then combined by averaging data points as a function of distance from the optical position, thus minimizing source confusion.

The average flux per beam position as a function of distance from SU UMa is shown in Fig. 1. For comparison the measured antenna response of a point source is also given. A source was detected at the optical position with a flux corresponding to

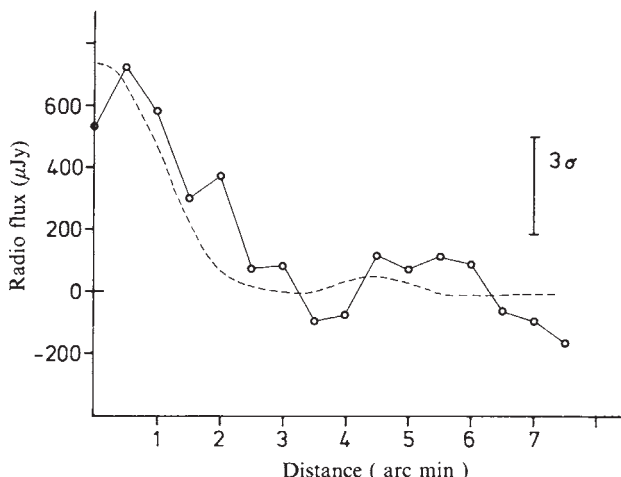


Fig. 1 Radio flux plotted against distance from optical source SU UMa. The antenna response of a point source (3C84) is shown for comparison.

Table 1 System parameters and observing periods

Frequency	4.75 GHz
System temperature	65 K
Bandwidth	600 MHz
Half power beam width	2.4 arc min
Observing periods (1982)	
22–23 April	Outburst (decline) 36 scans
13 June	Outburst (decline) 18 scans
25–27 June	Quiescence 69 scans

about 6 s.d. of the background noise at distance 3 arc min from SU UMa. The noise is close to the expected level from the receiver. Background sources seem to be weak and effectively smoothed out. The field is apparently not rich in extragalactic sources. The half power width of the detected source is close to the actual beam size. No corresponding peak in the polarized intensity could be detected, suggesting that linear polarization is $<30\%$.

In a second analysis we divided the observations according to the optical activity of SU MUa. The first two observing periods were obtained 1 or 2 days after the peak of optical brightness as determined from the visual light curve of SU UMa supplied by the American Association of Variable Star Observers (AAVSO). Similar to Fig. 1, Fig. 2 shows two radial intensity profiles of SU UMa corresponding to the outburst and quiescence observations. Although the noise properties at distances beyond 2 arc min are comparable during the two states, a peak flux of 1.3 mJy is found at the position of SU MUa only during outburst. The probability of a chance coincidence with a source of 1.3 mJy or more is about 10%. However, the radio flux dependence on optical activity suggested by Fig. 2 strongly supports the idea that the detected emission during peak optical brightness can be attributed to SU UMa.

X-ray observations of SU UMa in quiescence² yield an emission measure of $7.6 \times 10^{54} \text{ cm}^{-3}$ in the 0.1–4.5 keV band assuming a temperature of 10^8 K and a distance of 220 pc (ref. 3). Independently of whether this hot plasma is optically thick or thin to radio waves, the estimated free-free emission turns out to be more than 3 orders of magnitude lower than the observed flux. Alternatively, the mass loss observed in UV lines of dwarf novae in outburst may be expected to emit free-free emission at a temperature of a few 10^4 K . The radio flux was calculated following the derivation of ref. 4. For an outflow velocity of $3,000 \text{ km s}^{-1}$ and a mass loss rate of $10^{-11} M_\odot \text{ yr}^{-1}$ observed in similar systems⁵, the calculated flux is 7 orders of magnitude below the observed value. It is therefore more reasonable to propose suprathermal electrons as the origin of the radio emission of SU UMa.

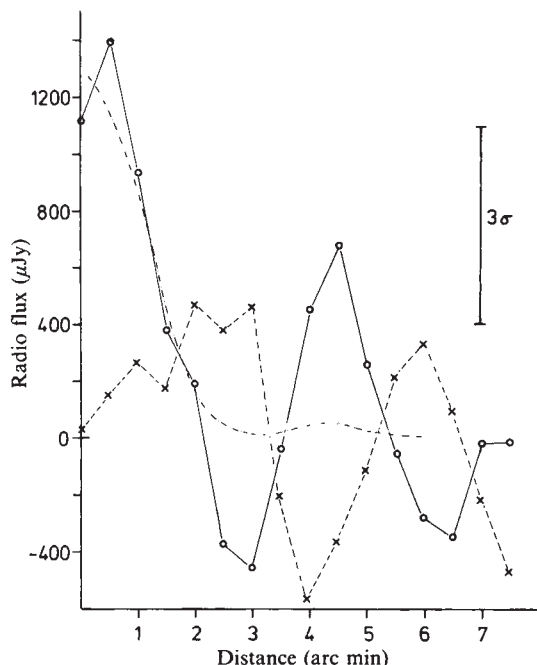


Fig. 2 Radio flux for outburst (solid curve) and quiescent (dashed curve) periods of SU UMa as a function of distance from the optical source. ○—○, 22–23 April + 13 June; ×—× 25–27 June; — · — ·, response of 3C84.

Gyro-synchrotron emission has recently been considered for the radio emission of the magnetic variable AM Her⁶. The white dwarf of a dwarf nova system is expected to have lower magnetic fields than in AM Her like binaries. If we apply the proposed mechanism to SU UMa, the source dimension turns out to be large compared with the binary distance, and magnetic confinement is unlikely. The expansion of the cloud of energetic electrons, following from this result, may be connected to the mass loss observed in UV. The radio flux modulation with optical outbursts then puts an upper limit on the source radius of a few travelling days or 10^{14} cm. A detailed analysis is necessary to show whether a synchrotron model is possible or not.

As an alternative to synchrotron radiation a coherent process may be considered. In this case the ratio of plasma frequency to gyrofrequency is an important parameter. Very little is known at present about this source parameter, and it is not reasonable to propose a detailed model. However, the cyclotron maser instability seems to be an attractive possibility. For a maser efficiency of 0.1%, the same order as in a well-known model of terrestrial auroral kilometric radiation⁷, the total energy requirement for suprathermal electrons would be only 10^{48} erg s⁻¹. This is 7 orders of magnitude below optical luminosity during outburst.

Synchrotron emission is linearly polarized and decreases in flux with higher frequency according to a power-law. Maser spectra are expected to have smaller bandwidths (a few octaves), and their emission is predicted to be circularly polarized. Future observations may decide whether the radio emission originates from an expanding synchrotron source or a cyclotron maser near the white dwarf.

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Evidence for the detection of X-ray scattering from interstellar dust grains

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Overbeck¹ has shown that small angle scattering of X rays by interstellar dust will tend to increase the apparent size of a point celestial X-ray source, the result being the formation of a halo around the source image. Although the exact description of this halo, which depends on the detailed properties of the dust and of the source, has been discussed^{2–6} no direct observational evidence for the existence of such haloes has been presented, although dust scattering has been invoked to explain the diffuse soft X-ray emission observed around the Crab Nebula during a lunar occultation⁷. I report here data obtained by the imaging proportional counter on board the Einstein Observatory⁸ of the bright X-ray source GX339–4 (4U1658–48). These data show a faint but apparently real X-ray halo around GX339–4. The principal uncertainty in the intensity distribution of the halo arises from the poorly determined spectrum of GX339–4 and it is likely that the parameters of the halo and of the derived model of the scattering dust grains may be improved in a future analysis using revised Einstein spectral calibration data; however, the range of allowed spectra leaves the reality of this first detection of an interstellar dust halo unchanged.

On 8 March 1979 the Einstein Imaging Proportional Counter (IPC) observed the region centred on the bright galactic source GX339–4 for ~1,700 s, during which time about 1.7×10^5 counts were collected in the energy range $0.2 < E(\text{keV}) < 4.0$. The image clearly shows the presence of one bright point-like source with no other field sources visible. Spectral information from IPC was available in 16 pulse height (PH) channels, most of the recorded events occurring in channels 5 and above. Because the counting statistics were very good this range has been divided into two for subsequent analysis. The low-energy range was defined to be PH channels 5–9, and the high-energy range to be PH channels 10–15, corresponding to the photon energy ranges $0.5 \leq E(\text{keV}) \leq 1.8$ and $1.8 \leq E(\text{keV}) \leq 4$ respectively.

The background flux for the image data was estimated from within an annulus of radii 25–30 arc min centred on the overall field of view and sufficiently far from the GX339–4 to render its contribution negligible. Both particle and soft X-ray background fluxes have been allowed for, the latter being modified by the telescopes vignetting function and the IPC's window support⁶.

Before launch the Einstein Observatory was subjected to a comprehensive series of calibration tests conducted at the Marshall Space Flight Center's long beam test facility. Data from

Table 1 Observed fractional excess flux in an annulus from 3 to 15 arc min from the image centroid for GX339–4

Spectrum	Fractional excess		Significance of data	
	Low	High	Excess above prediction	(σ)
	$0.5 \leq E(\text{keV}) \leq 1.8$		$1.8 \leq E(\text{keV}) \leq 4$	
	(%)	(%)	Low	High
IPC	5.5	3.9	39	21
MPC	3.4	1.9	25	12

The finite energy resolution of the IPC should be borne in mind whilst considering the above figures ($\Delta E/E \sim 1$ for $E \leq 1.5$ keV, $\Delta E/E \sim 2$ for $E \sim 0.25$ keV).

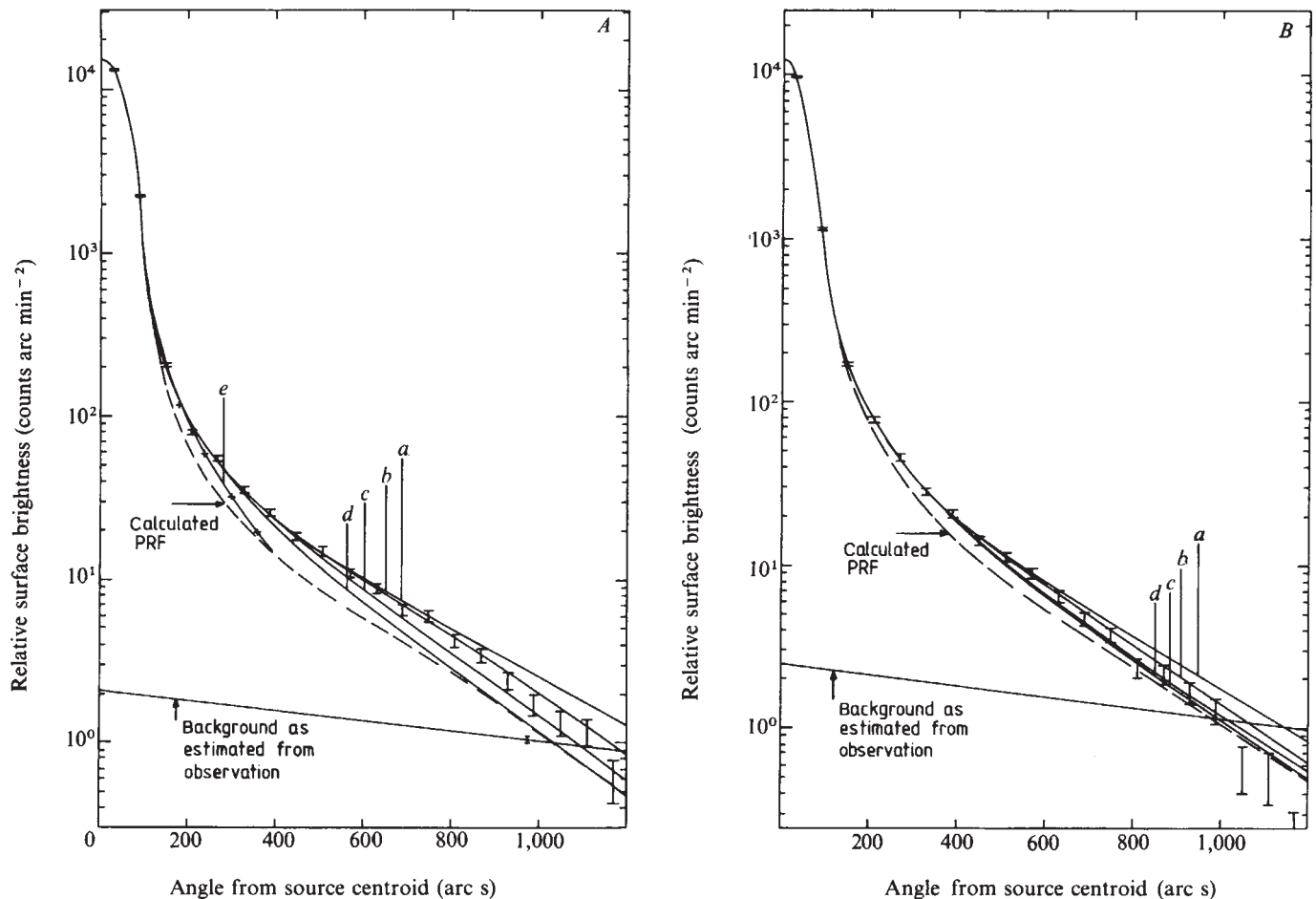


Fig. 1 Radial surface brightness distributions of the X-ray image of GX339-4 in the A, low (5-9), and B, high (10-15) energy channels are compared with the calculated distributions for interstellar dust scattering, with a range of single-size grain models. The Einstein-IPC point response function (PRF) calculated from pre-flight calibration data is also shown for each energy band. *a*, $\alpha = 0.04 \mu\text{m}$; *b*, $\alpha = 0.06 \mu\text{m}$; *c*, $\alpha = 0.08 \mu\text{m}$; *d*, $\alpha = 0.1 \mu\text{m}$; *e*, $\alpha = 0.32 \mu\text{m}$.

these tests (F. D. Seward, personal communication) were used to model the IPC's point response function (PRF) as a function of incident X-ray energy, detector PH channel and high voltage settings. The latter two functions ensured that any gain changes experienced by the IPC could be accounted for in the analysis. The effect on the PRF of having the source at small off-axis angles was also examined. All data were radially summed so that the IPC PRF wings could be described accurately out to 15 arc min from the source centroid. These PRFs were described by a series of gaussian functions and the results incorporated into a computer code which allowed the expected IPC response to be calculated for a near-axis point source of known X-ray spectrum, given specific IPC gain conditions (these were determined in flight before the observation).⁶ A requirement for the gaussian fitting procedure was that all results gave a reduced χ^2 statistic of < 1 .

Before the actual comparison of a point source with the data could be made the intrinsic spectrum of GX339-4 had to be determined. Unfortunately, the IPC data are not well fitted by a simple power law (thermal fits are much worse still), the best fitting version being of the form

$$\frac{dN}{dE} \propto E^{-7.5} \exp\left(-\frac{2.0}{E}\right)^{8/3}; \quad \chi^2_{\nu} \approx 12$$

The poor fit may be due in part to the very high statistical quality of the data and the fact that no error on the instrumental calibration has been included. Another measure of the source

spectrum was obtained by the Einstein Monitor Proportional counter (MPC) at the same time. This gave:

$$\frac{dN}{dE} \propto E^{-3.2} \exp\left(-\frac{1.92}{E}\right)^{8/3}; \quad \chi^2_{\nu} \approx 20$$

(Both the spectra agree that the low-energy cutoff occurs at close to 2 keV.) When this is folded through the IPC's spectral response a value of $\chi^2_{\nu} \approx 24$ was obtained. This uncertainty in the exact spectral form gives the largest error in the analysis.

To show the existence of an excess of scattered flux in the image a comparison was made between the flux in an annulus from 3 to 15 arc min in radius (centred on the peak in the image distribution) to the total flux within 15 arc min radius, for both the image data and the predicted PRF, in the two PH ranges. Using the computer code to compare the image results with the ground test data, for both the IPC and MPC spectral forms, showed that in both cases the GX339-4 image could not be explained by a point source alone, but required the additional presence of a faint diffuse component (see Table 1 and Fig. 1). Varying the assumed spectral form from the IPC and MPC, such that the reduced χ^2 statistic increased by more than a factor of 2 above that of the MPC fit, changed the significance of the detection only and not its reality. An increase in the low energy cutoff by more than 0.5 keV was necessary to reduce the detection significance below 5σ . This excess may be explained in one of three ways; either in-flight instrumental

degradation, or intrinsic source extension, or the result of X-ray scattering by interstellar dust.

Image data from the Einstein High Resolution Instrument of 3C273 (R. Willingale, personal communication) have shown the performance of the telescope mirrors to be as during ground testing, essentially ruling out additional scattering due to an increase in mirror surface roughness (the energy dependence

Table 2 The best fit results for spherical silica grains based on a uniform dust distribution and constant source intensity model

$n(\alpha)$	α_0 (μm)	Low energy χ^2_ν (for best fit)	High data	Grain column density $N(\text{cm}^{-2})$	Spatial grain density $n_g(\text{cm}^{-3})$
$(\alpha = \alpha_0)$	0.06	1.5	2.3	3.6×10^{10}	0.6×10^{-11}
$\exp[-5(\alpha/\alpha_0)^3]$	0.11	1.2	1.4	8.9×10^{10}	1.5×10^{-11}

The value of n_g is calculated assuming a distance of 2kpc to GX339-4. The results for N and n_g can be scaled for other chemical species by dividing them by the square of the ratio of the electron density of the new chemical species to that of silica (SiO_2) (ref. 6). The size results are independent of grain chemistry.

is the opposite to that observed in any case). There is no suitable in-flight calibration of the IPC itself to verify its performance directly. However, a critical evaluation of the image data in individual PH channels showed no unexpected charge-dependent effects, even in the lowest channels.

Of the remaining possibilities, the identification of a stellar optical counterpart for GX339-4 (refs 9, 10), together with measurement of ~ 40 ms time variability¹¹, indicate that a point-like nature for the source is highly probable. We therefore conclude that the observed excess in X-ray flux is due to scattering of X rays by interstellar dust.

A first simple check of this conclusion may be made by assuming that the grains responsible for the X-ray scattering are also those which cause the optical extinction. The X-ray scattering optical depth may then be related to the total optical extinction, A_v , and the efficiency factors for X-ray scattering, Q_s , and optical extinction, Q_e , by²

$$\tau_s = \frac{A_v Q_s}{1.086 Q_e}$$

Typically, Q_e has been taken to be 1.5 (ref 12). For spherical silicate grains of radius $0.1 \mu\text{m}$, $Q_s \approx 0.02$ at an X-ray energy of 2 keV which, with the measured value of A_v (≈ 3.75) for GX339-4 (ref. 10), gives $\tau_s \approx 0.05$, or a fractional halo flux of 5%. This is in good agreement with the measured excess (which does, however, ignore the contribution from within 3 arc min).

The statistical quality of the data warranted a more detailed analysis in terms of the halo's radial brightness function, the theoretical description of which has been given elsewhere^{4,6}. The overall shape of the halo is a function of the spatial distribution and physical properties of the dust (grain shape, orientation and size distributions) and the wavelength of observation. The grain chemistry only affects the intensity of scattered X rays. However, variations in the source intensity and spectrum which occurred before the observation will, due to the pathlength difference between scattered and unscattered X rays, be spread through the observed halo^{6,13}. Unfortunately,

no information was available on the behaviour of GX339-4 before this Einstein observation and so any results achieved by this detailed analysis may be subject to error. For a distance of 2 kpc (which is a lower limit based on the value of A_v)¹⁰ intensity and spectral data for ~ 10 days leading up to the halo observation would be necessary to allow an accurate description to be made.

A simple model of a cylindrically symmetrical uniform distribution of spherical grains about a line of sight to a constant source was adopted. Haloes were then calculated by a specially written computer code, added to a point source, and then convolved with the instrumental imaging and energy responses. For this part of the analysis the IPC spectrum was used and the best fitting results, considering two types of grain size distribution (a single size and a Greenberg size spectrum)¹², are given in Table 2. Figure 1 compares the single size grain model results with the calculated radial PRF and GX339-4 data. The spatial form of the Greenberg size spectrum halo can be closely approximated by that of a single size model since particles of radius $\alpha = 0.64\alpha_0$ (α_0 is the Greenberg spectrum cutoff radius) dominate in the total scattering cross-section. (In fact for $\alpha = 0.45\alpha_0 \rightarrow 0.85\alpha_0$ the total scattering cross-section varies by only a factor of 2.) Incorporating variations in the source intensity on time scales ~ 1 day and by factors ≤ 5 had no effect on the results for grain size, but only on the number of grains needed to provide the observed amount of scattering. This is due to the limited spatial resolution of the IPC (~ 1.5 arc min) which tends to blur out these effects even more than is caused naturally in the halo and renders them unobservable. However, the grain number density is affected as the halo flux relative to that of the source is then incorrectly estimated from the observation.

Mathis *et al.*¹⁴ have found that the optical extinction can be explained, for various grain chemistries, if the number of grains with radius α is described by $n(\alpha) \propto \alpha^{-3.5}$ ($0.25 \geq \alpha(\mu\text{m}) \geq 0.025$). Using this form with the present data over suitably restricted size limits showed that the X-ray data were not consistent with such a simple power law. Instead the data required the power law index to be < 4 for grains with $\alpha \geq 0.1 \mu\text{m}$. A power law index of exactly -4 ensures that the halo flux is independent of grain size.) These larger grains give rise to narrow haloes which would tend to broaden the core of the image (where the counting statistics are good). For the smaller grains ($\alpha < 0.05 \mu\text{m}$) which produce broader haloes, the data are not very sensitive to changes in power law index (as the counting statistics become poorer in the image wings) and a value of ≈ -3.5 is acceptable. Note that this appears to describe a size distribution with an upper grain cutoff radius of around $0.1 \mu\text{m}$ and possibly a levelling out in the variation of grain number versus radius for the smaller grains ($\alpha < 0.05 \mu\text{m}$).

Clearly, from the above initial results, the potential of X-ray scattering measurements on the study of interstellar dust appears good. However, it must be stressed that this was the only source observed by the Einstein Observatory which was bright enough to allow this analysis to be performed. Future observations are essential both to confirm these present data and to allow the full potential of X-ray scattering as a means of examining the nature of the interstellar dust to be realized.

As the Einstein observatory is now inoperative the next opportunity to develop this potential will arise with the launch of the European EXOSAT X-ray astronomy satellite this year.

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Detection of two silicon environments in kaolins by solid-state ^{29}Si NMR

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An investigation of kaolins using solid-state ^{29}Si NMR and X-ray crystallography is being carried out to study relationships between measured NMR parameters and X-ray determined properties. We report here some preliminary results of the ^{29}Si NMR examination which provide new information on the structure of kaolins. Lippmaa and co-workers¹ reported a single ^{29}Si resonance for kaolinite at 39.75 MHz consistent with the existence of only one type of chemical environment for silicon as expected. We have examined highly crystalline samples of the three forms of kaolin by ^{29}Si NMR at 59.61 MHz and resolved this peak into two components. This splitting of the ^{29}Si resonance seems to be rationalized only by the presence, in fact, of two slightly different silicon environments in kaolins.

Kaolins ($\text{Al}_4[\text{Si}_4\text{O}_{10}](\text{OH})_8$) have a $Q^3(\text{OAl})$, dioctahedral repeat '1:1' layer structure consisting of an octahedral aluminium hydroxide sheet (gibbsite sheet) and a tetrahedral silica sheet². Bonding between the gibbsite and silica sheets is through the apical oxygen of the silica tetrahedra replacing one hydroxyl group of the gibbsite sheet. A rigid three-dimensional structure is obtained through hydrogen bonding between the silica sheet basal oxygens of one layer and the hydroxyl groups of the gibbsite sheet of the adjacent layer. Three forms of chemically identical kaolin occur: kaolinite, dickite and nacrite, with differences being in the stacking of layers in different regular sequences. Kaolinite has a single-layered triclinic unit cell, dickite and nacrite have a two-layered monoclinic cell².

In several recent papers, solid-state ^{29}Si NMR has been used to study silicates, aluminosilicates, zeolites and clay fractions^{1,3-8}. The utility of this technique for obtaining structural information was first demonstrated by Lippmaa and co-workers^{1,3,4}. Increasing degree of silica tetrahedra condensation was found to lead to increased diamagnetic shielding whereas substitution of aluminium for silicon results in paramagnetic deshielding. Linewidths were found to be sensitive to the regularity of Si and Al distribution in aluminosilicates. Lippmaa and co-workers have examined several layered aluminosilicates, including kaolinite and pyrophyllite^{1,4}, by solid-state ^{29}Si NMR at 39.75 MHz. For kaolinite, a single resonance at -91.5 p.p.m. was reported¹ which is consistent with the presence of a single $Q^3(\text{OAl})$ silicon environment. Pyrophyllite, while also being a $Q^3(\text{OAl})$ aluminosilicate like kaolin, differs in that it has a 2:1 dioctahedral structure with a silica tetrahedral sheet on either side of the gibbsite sheet. Lippmaa⁴ has reported a ^{29}Si resonance for pyrophyllite at -95 p.p.m. (a second resonance at -91.5 p.p.m. was reported, which we believe to be due to a kaolin impurity as found for the sample of pyrophyllite we have examined), again consistent with a single $Q^3(\text{OAl})$ silicon environment. The difference in shift between kaolin and pyrophyllite can be rationalized according to the differences in structure. The hydrogen bonding present in kaolin, which results in a separation of ~0.7 nm between the aluminium or silicon layers of adjacent sheets, is absent in pyrophyllite, leading to a separation of ~1.4 nm. Hence the inter-layer hydrogen bonding seems to cause a small deshielding in kaolin compared with pyrophyllite.

We have examined a range of kaolins of different X-ray determined crystallinities, but in all cases a resonance was

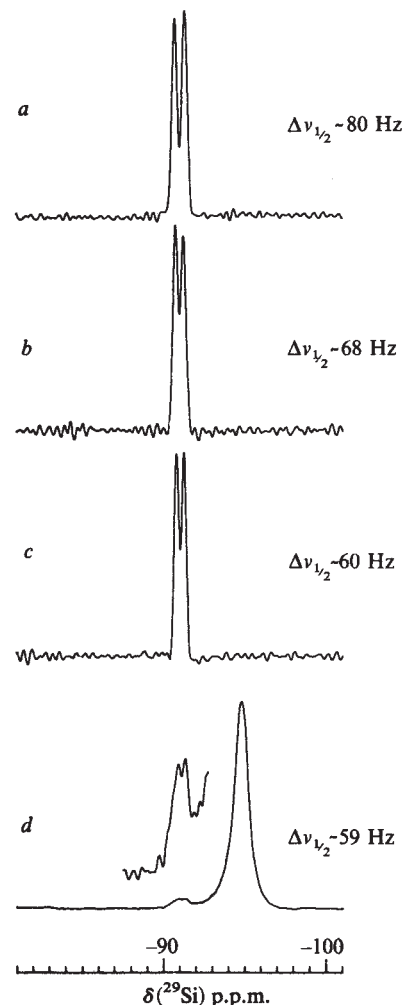


Fig. 1 ^{29}Si CP/MAS spectra of: *a*, kaolinite; *b*, nacrite; *c*, dickite; and *d*, pyrophyllite. Spectra were obtained at 59.61 MHz on a Bruker CXP-300 spectrometer using ^1H and ^{29}Si ^1H fields of 10 and 50 G respectively, single 5-ms contacts and a recycle time of 3 s. Spectra shown in *a-c* are gaussian resolution-enhanced. Samples were packed in Delrin rotors, spun at ~3.5 kHz and contained a small quantity of KBr used to adjust the magic angle and check rotor stability through observation of the ^{79}Br resonance.

obtained at -91 p.p.m., in good agreement with the previous results. Linewidths were accurately and reproducibly determined using ^1H decoupling fields of >10 G and accurate setting of the magic angle on each sample, and were found to vary from ~60 to >110 Hz. However, careful inspection of spectra of samples having linewidths of ≤ 90 Hz revealed a splitting of the resonance into two peaks of approximately equal intensity. Figure 1*a-c* shows resolution-enhanced ^{29}Si CP/MAS spectra of samples of each of the three forms of kaolin which clearly reveal the presence of two overlapping resonances. This apparent doublet is evident in unenhanced spectra to a variable extent depending on the size of the chemical shift difference and the overall linewidth. The shift difference varies from ~27 Hz (0.45 p.p.m.) in kaolinite ($\Delta\nu_{1/2} \sim 80$ Hz) to ~17-19 Hz (0.29 p.p.m.) in nacrite ($\Delta\nu_{1/2} \sim 68$ Hz) and dickite ($\Delta\nu_{1/2} \sim 60$ Hz). Identical splittings and relative intensity were obtained from single pulse experiments with dipolar ^1H decoupling. Studies of cross-polarization and relaxation behaviour indicated that both peaks in all samples behaved identically. However, significant differences were observed between samples and these will be discussed elsewhere.

There are two possible explanations for this splitting. First, it could arise from ^{29}Si - ^{27}Al dipolar interaction as has been

observed for ^{13}C directly bonded to ^{14}N in solid-state ^{13}C spectra obtained at low field⁹⁻¹³. The effect and splitting have been observed to decrease with increasing field strength due to increased Zeeman splitting^{10,13}. This possibility can be virtually discounted as no similar splitting has been reported for the wide range of aluminosilicate minerals and zeolites previously examined^{1,3-7}. For example, linewidths of <2 p.p.m. at 39 MHz have been reported by Lippmaa and co-workers^{1,3-6} and no splittings have been observed even though such an effect would be larger at the lower field. As an example of this, a spectrum of pyrophyllite (Fig. 1d) reveals no splitting even though a linewidth of only 59 Hz is observed. Note that a weak signal at -91 p.p.m. with a splitting of ~27 Hz is present, indicating a small impurity of kaolin in this sample.

The other explanation is that two different but equally populated silicon sites exist in the kaolin structure. The shift difference of <0.5 p.p.m. would indicate that the difference between environments is only very slight. No unequivocal evidence exists from other sources to support this conclusion. However, X-ray diffraction can only provide limited information on bond lengths and angles within the silicon-aluminium layers. The presence of two slightly different silicon environments is probably due to the distortion within the layer and perhaps partly as a result of the need to accommodate interlayer

hydrogen bonding. No such splitting is observed in the 2:1 species, pyrophyllite, possibly due to the presence of the second silica layer or the absence of the interlayer hydrogen bonding reducing the distortion. Furthermore, the variation of the chemical shift difference in the three forms of kaolin may be a consequence of subtle differences in hydrogen bonding due to the different layer packing¹⁴. For example, the splitting in dickite and nacrite is <20 Hz while for kaolinite it is ~27 Hz, perhaps implying greater distortion to accommodate hydrogen bonding in the latter. Shift differences of this small magnitude have been observed by Lippmaa and co-workers⁴ for the trimethylsilyl ester of double four-ring octameric silicate, Q_8M_8 , due to slight distortion of the cube in the crystal lattice.

These results again demonstrate the value of solid-state ^{29}Si NMR in structural investigation of solid silicates and aluminosilicates. In particular, the results provide important new information on the structure of kaolins. Our investigations of kaolins and other clay minerals by solid-state ^{29}Si NMR are continuing. The final interpretation of these new data from ^{29}Si NMR remains a challenging problem for crystallographers and mineralogists.

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Ophiolitic mélange separates ortho- and para-tectonic Caledonides in western Ireland

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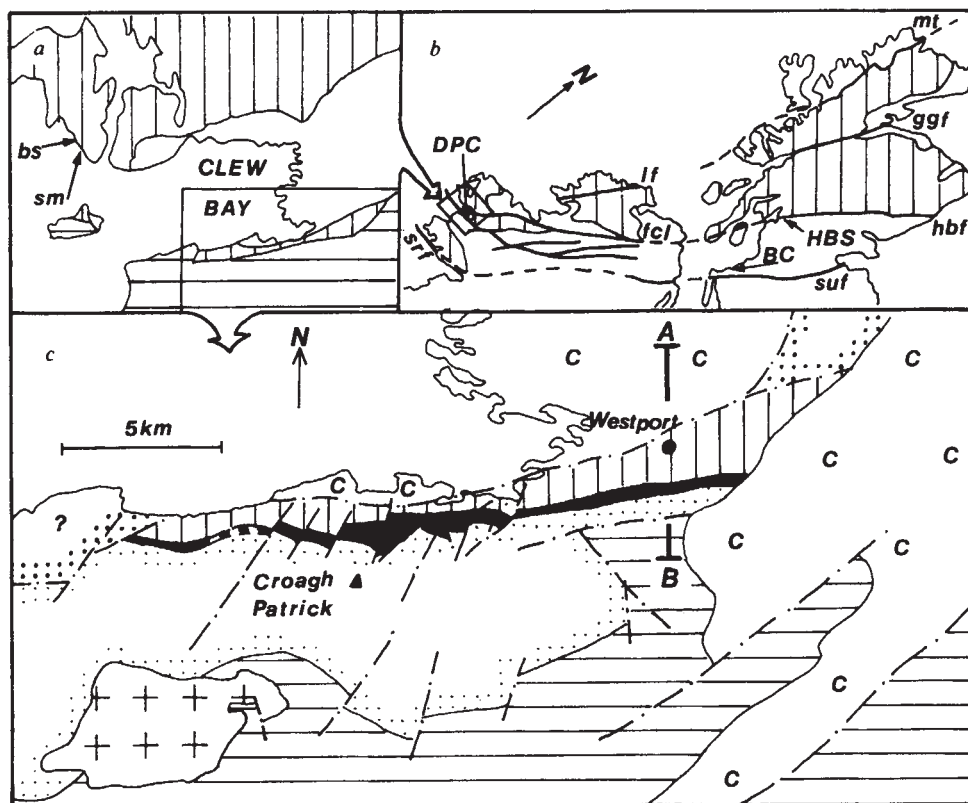
The nature of the contact between the orthotectonic (late Pre-cambrian to Cambrian) and the paratectonic (Ordovician and Silurian) Caledonides has long been a source of discussion. In western Ireland this junction occurs in a complex zone which crops out along the southern shores of Clew Bay, County Mayo, where Dalradian (Middle Cambrian) and Ordovician (Arenig) rocks are separated by a pre-Silurian rock unit termed the Deer Park Complex¹ (Fig. 1). The complex contains both meta-sedimentary and meta-igneous rocks and has been variously interpreted as representing a pre-Caledonian basement ridge¹, an imbricate thrust zone² related to the development of a major fault system, the Fair Head-Clew Bay line³ or an Arenig ophiolite⁴. We report here geological, geochemical and geophysical studies on the Deer Park Complex which indicate that it is a northerly dipping mélange unit containing the components of a dismembered ophiolite. These findings imply a source of ophiolitic rocks to the north possibly within the orthotectonic Caledonides.

The Deer Park Complex occupies a zone of between 0.1 and 1.1 km in width that can be traced for 18 km along strike from Westport westwards onto the northern slopes of Croagh Patrick (Fig. 1c). Outcrop is discontinuous with small knolls, mainly composed of relatively unshaped meta-igneous rocks, being surrounded by poorly exposed lower ground that contains more

highly deformed, locally mylonitic, material. There is no obvious sequence in the unshaped material, although metasediments are most common in the central region. Trenching has shown that the lower ground is often occupied by talc-carbonate schists (D. McDougall, personal communication). In the central region the Deer Park Complex is in tectonic contact with Dalradian rocks attributed to the Southern Highlands Group¹. This contact has been interpreted as a Grampian slide¹. The nature and timing of the contact with the Ordovician to the south are unknown as it is everywhere covered by unconformably overlying Silurian strata.

The following lithologies are recorded in the complex: metasediments, carbonate-talc-tremolite schists, amphibolites, serpentinites, metagabbros, metapyroxenites, metadolerite, serpentinite breccia and rodingites. A brief description of their petrology and petrochemistry follows; detailed results will be published elsewhere (V.K.S., in preparation). The metasediments are dominantly semi-pelitic, display a polyphasal deformational history and contain assemblages typical of the epidote-amphibolite facies (garnet-biotite-muscovite-oligoclase/andesine). In the carbonate-talc-tremolite schists serpentinite has been replaced by magnesite with some associated calcite and dolomite. The striped amphibolites are fine grained and contain actinolitic hornblende-albite/oligoclase-epidote assemblages and may represent metavolcanics. Chromite layering is the only primary texture preserved in some of the serpentinites. Olivine is replaced by antigorite and pyroxene by bastite and less frequently actinolitic hornblende with a low Al (VI) content indicating a low pressure of formation^{5,6}. Veinlets of chrysotile are common throughout. The CIPW normative mineralogy²¹ places the serpentinites within the dunite-hartzburgite fields, which is in agreement with their low Al_2O_3 contents (0.24 wt%). Their Ni (2,100-2,400 p.p.m.) and Cr (1,400-1,600 p.p.m.) contents are within the field of Alpine peridotites⁷. The chromite has altered to spinel except in the

Fig. 1 *a*, Outline geological map of the Clew Bay area showing the location of the orthotectonic Caledonides (vertical ruling) and the paratectonic Caledonides (horizontal ruling). The location of blueschists (bs) and serpentinite mélanges (sm) on Achill Island are shown. *b*, Geological map of the northern British Caledonides showing the regional distribution of the orthotectonic Caledonides (vertical rule). The location of Caledonide ophiolitic complexes (DPC, Deer Park Complex; BC, Ballantrae Complex; HBS, Highland Border Complex) and major fault lines (FCI, Fair Head-Clew Bay line; GGF, Great Glen Fault; HBF, Highland Boundary Fault; LF, Leenan Fault; MT, Moine Thrust; SRF, Skird Rocks Fault; SUF, Southern Uplands Fault). *c*, Geological map of the Deer Park Complex (black) showing its position in relationship to the Dalradian (vertical ruling), the Ordovician (horizontal ruling), the Silurian (small dots) and the Devonian (large dots); C, Carboniferous; crosses, granite. The line of section A-B of Fig. 2 is also shown.



core zones which have a low TiO_2 content (0.03–0.19 wt%) and span a range of $\text{Cr}/(\text{Cr} + \text{Al})$ and $\text{Mg}/(\text{Mg} + \text{Fe}^{2+})$ ratios (36–76 and 58–65 respectively) typical of chromites from Alpine peridotites⁸. Hydrogrossular bearing rodingites occur in veins which cross cut both serpentinites and amphibolites.

The metagabbros and metadolerites preserve ophitic texture. In one knoll (mr L913812) the metadolerites display the one-sided chilled margins characteristic of sheeted dykes. Eight such dykes of 75 cm mean thickness are recorded. These metabasites contain remnants of sub-calcic augite, twinned saussuritized plagioclase (An_{30-34}), chlorite, zoisite-clinozoisite, albite, actinolite with variable amounts of sphene and magnetite. The chemical composition of the dolerites indicates that they are altered tholeiites (qz normative, $\text{TiO}_2 \times 100/\text{Zr} = 140\text{--}170$) and in terms of their Ti (1.05–0.5 wt%), Zr (69–36 p.p.m.), Y (50–29 p.p.m.) and Cr (246–168 p.p.m.) contents are characterized as ocean floor basalts ($\text{TiO}_2 \times 100$, Zr, Y $\times 3$ (ref. 9) and Ti, Cr (ref. 10)) and mid-ocean ridge basalts (MORB) overlapping the field of island arc tholeiites (Ti, Zr (ref. 11) and Zr, Y (ref. 12)). However, on the Cr, Y plot¹¹ they are characterized as MORB, not island-arc tholeiites. The serpentinite breccias contain irregular (maximum 20 cm) clasts of serpentinite and pyroxenite, and grains of pyroxene and chromite in a serpentinite matrix. These rocks differ from the massive serpentinites in that both clasts and matrix have a higher Al_2O_3 content (6.38–16.16 wt%). No primary bedding is visible in these breccias which apparently occur as pipes within the massive serpentinites.

Gravity measurements were made along two approximately south–north profiles across the Deer Park Complex. One profile passed through Westport and comprised 158 stations over a total distance of 10 km (Fig. 1c). An asymmetric residual Bouguer anomaly of amplitude +22 gravity units was observed which had a sharp rise close to the southern contact of the complex and a more gradual tail to the north. A similar shape was recorded along a parallel but less complete profile 14 km to the west. Computer modelling using a two-dimensional gravity program¹³ suggests that the complex occurs as a slab-like body which dips northwards at $\sim 45^\circ$ and has an irregular

cross-section (Fig. 2). The maximum depth to which the body can be modelled is 2.6 km and it has to be assumed that either the body terminates at that depth or that the density contrast decreases with depth. A positive density contrast of 230 kg m^{-3} (130 kg m^{-3} for the near-surface part) was assumed for the model. Although more density readings are needed for this inhomogeneous complex the general conclusions would not be altered even by quite large changes in density.

The geophysical findings coupled with the discontinuous outcrop, the presence of a pervasive shear fabric and the juxtaposition of such a variety of rock types all suggest that the complex has been disrupted along a northerly dipping thrust zone. The attitude of this zone suggests a component of tectonic transport from the north whilst a horizontal stretching fabric in the overlying Silurian conglomerates indicates subsequent reactivation in a strike-slip regime. The nature of the outcrop is such that it is not possible to tell whether this complex represents a true mélangé or a sheared olistostrome. The metabasic and ultramafic rocks show compositional and textural features consistent with their being derived from an ophiolite which has been dismembered either during emplacement or before this event.

The hypothesis that the Deer Park Complex represents a dismembered ophiolite occupying a major north dipping shear zone is inconsistent with the earlier interpretation of this complex representing a basement ridge of Lower Dalradian, Moian or Grenvillian age¹. Thus this zone must now be interpreted as marking a major crustal break involving plate destruction which may have been associated with the early formation of Grampian blueschists¹⁴ within the Dalradian of North Mayo (Fig. 1a). Furthermore, arguments concerning the age of the Grampian event¹ based on the correlation of early structures across this zone must now be questioned. The juxtaposition of Southern Highland Group rocks with an ophiolitic complex during the Grampian orogeny is difficult to reconcile with a totally ensialic Dalradian sedimentary basin¹⁵, especially when coupled with the nearby occurrence of serpentinite breccias in the Dalradian of Achill Island¹⁶ (Fig. 1a) and the nature of the tholeiitic sills within the Dalradian of Scotland¹⁷. The

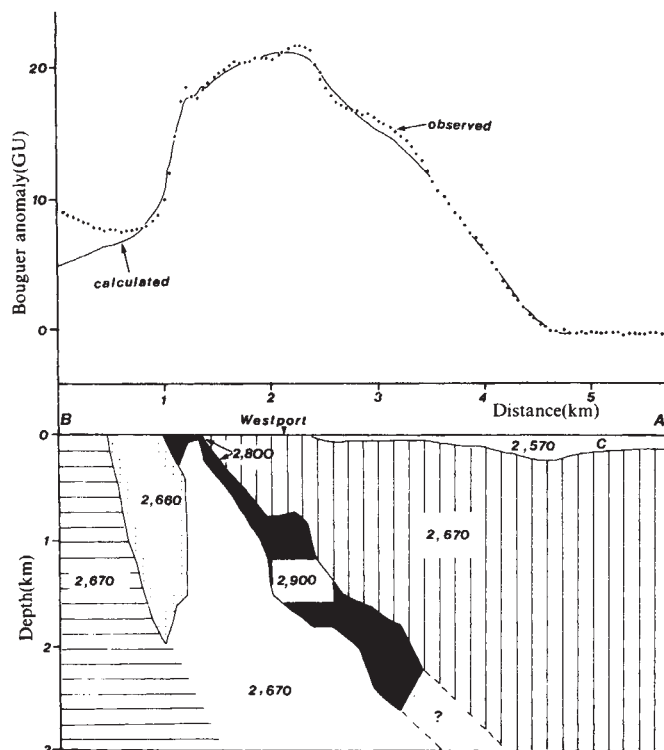


Fig. 2 Gravity (Bouguer Anomaly) profile along the approximate north-south line (A-B) through Westport showing observed anomaly and its fit to the calculated anomaly for the model shown beneath. Key as in Fig. 1c, densities given in kg m^{-3} .

inferred component of tectonic transport from the north is not consistent with the suggestion⁴ that the gabbros and serpentinites within this complex represent basement to the primitive island rocks of the Tremadoc-early Arenig Lough Nafuoey Group¹⁸ which lie 50 km to the south, unless this basement was more extensive than presently supposed. Alternatively the Deer Park Complex may have had its origin in a separate sedimentary basin from within the orthotectonic zone. These last two models are similar to those proposed for the Highland Border Series of Scotland^{19,20}, which shows many apparent lithologic and tectonic similarities with the Deer Park Complex. It is not possible to reject any of these speculative models until the precise age of this complex is known. Finally, the findings of this study support the view that the Fair Head, Clew Bay Line (Fig. 1b)³ represents a major crustal break in the Irish Caledonides.

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Magma genesis and chamber processes at Los Humeros caldera, Mexico—Nd and Sr isotope data

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The Mexican volcanic belt (MVB), a roughly east-west structure, consists of many late Tertiary and Quaternary cindercones, domes, calderas and stratovolcanoes^{1,2}. Los Humeros caldera (approximately 19°40' N latitude, 97°25' W longitude) lies on the northeastern part of the MVB where the belt overlaps with another major volcanic province, the Eastern cordillera³ (Fig. 1). A compilation⁶ of the bulk chemical analyses of the two major volcanic provinces indicates that the MVB is characterized largely by calc-alkaline series whereas rocks of the alkaline series dominate the Eastern cordillera (EC). Pleistocene to Recent basaltic to rhyolitic volcanism in Los Humeros caldera, one of the best known examples of a well-developed caldera in Mexico⁷⁻⁹, presumably associated with the subduction of Cocos plate along the Middle America trench, shows that the initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios range from 0.7039 to 0.7048 and the initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratios from 0.5126 to 0.5129. We show here that these isotope ratios are negatively correlated and lie on the mantle array defined by MORB and oceanic island rocks; implying that Los Humeros magmas were generated in the upper mantle with very little, if any, contribution from the subducted oceanic crust, sediments or continental crust.

A large andesitic volcano was formed in Los Humeros during the Pliocene. The eruption of mainly felsic products (rhyolites, trachytes, and ignimbrites) seem to have partly emptied the magma chamber and caused the volcanic structure to collapse, thereby forming the caldera (H. Ferriz, personal communication)¹⁰. The present caldera is about 400 m deep and is irregularly circular with an approximate diameter of 16.5 km. Post-caldera activity consisted of the formation of numerous smaller volcanic cones and eruption of basaltic to andesitic magmas as well as deposition of sheets of basaltic to rhyolitic pumice and ashes⁷. Major element geochemistry of Los Humeros caldera shows the presence of both calc-alkaline and high-K calc-alkaline sequences, or both alkali and high-alumina basalt series^{9,10}. A preliminary geochemical study of alkali and alkaline earth elements and Sr isotopes of nine samples of basalt to andesite and one sample of high-K rhyolite shows the dominant role of fractional crystallization in the petrogenesis of Los Humeros caldera¹¹.

Measurements are now reported (17 new and 10 reported recently¹¹) of $^{87}\text{Sr}/^{86}\text{Sr}$ ratio as well as 11 new $^{143}\text{Nd}/^{144}\text{Nd}$ data on selected samples from Los Humeros caldera. The only previously reported Nd isotope data on MVB or EC are for lavas of Colima area¹² (westerly locations in the MVB). Sr and Nd isotope compositions were measured on a V. G. Micromass 30 mass spectrometer with an on-line computer and magnetic-field peak-switching capabilities. The experimental procedures are described elsewhere (ref. 13 and S.P.V., J.-G. Schilling and G. Waggoner, in preparation). K, Rb and Sr concentrations were determined using a mass spectrometric isotope dilution method described elsewhere¹³. Total procedural blanks (60 ng K, 0.1 ng Rb, 0.2 ng Sr and 0.2 ng Nd) were negligible for this work. The results of these measurements are given in Tables

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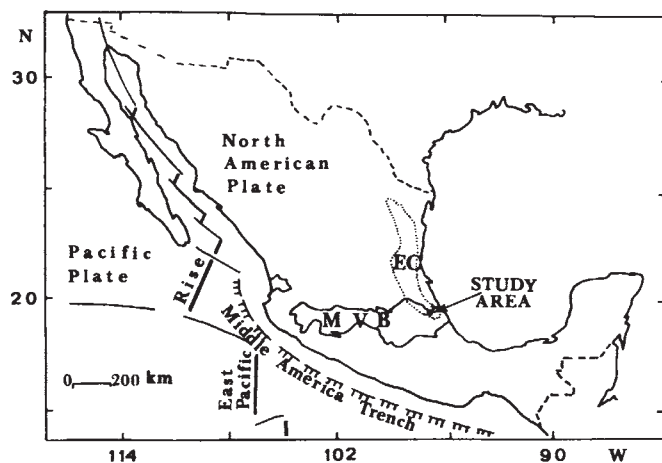


Fig. 1 A simplified map^{4,5} showing the location of the study area. MVB, Mexican volcanic belt; EC, Eastern cordillera³.

1 and 2. The post-caldera samples are all geologically very young and hence the $^{87}\text{Sr}/^{86}\text{Sr}$ and $^{143}\text{Nd}/^{144}\text{Nd}$ ratios reported in Table 2 can be taken as the initial ratios. Further, the measured $^{143}\text{Nd}/^{144}\text{Nd}$ ratios reported for the precaldra samples in Table 1 can also be taken as the initial $^{143}\text{Nd}/^{144}\text{Nd}$ ratios. However, for some pre-caldera samples especially those with high Rb/Sr, the correction for radiogenic ^{87}Sr growth may be significant. Unfortunately their emplacement ages are not yet precisely known except for sample CH42 (~1.2 Myr by K-Ar) (H. Ferriz, personal communication) which is the oldest sample reported here. The initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios are calculated for pre-caldera samples (Table 1) on the basis of an assumed age of 1 Myr. It can be seen from Table 1 that the initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio for only one sample is highly sensitive to the assumed value of the emplacement age.

The initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios for seven pre-caldera samples range from 0.7040 to 0.7048 (Table 1), whereas those for twenty post-caldera samples vary from 0.7039 to 0.7046 (Table 2). The average of all analyses is 0.7041_{-7}^{+4} (2σ ; $n = 27$). The correlation of the initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios with SiO_2 , K_2O , Rb, Sr, $1/\text{Sr}$ or $^{87}\text{Rb}/^{86}\text{Sr}$ is rejected at the 5% level of significance (the Pearson correlation coefficients, $r < 0.38$). The only possible exception at this level of significance may be for $^{87}\text{Sr}/^{86}\text{Sr}$ with SiO_2 (Fig. 2; $r = 0.377$, $n = 27$). It is interesting that this correlation becomes insignificant ($r \approx 0.27$) if the sample CH42 (which shows the highest initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio) is eliminated from the group. This may imply that this rhyolitic magma acquired a small sialic crustal component due to the interaction in the magma chamber with the surrounding crust: such a conclusion may also be compatible with the combined $^{143}\text{Nd}/^{144}\text{Nd}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ data.

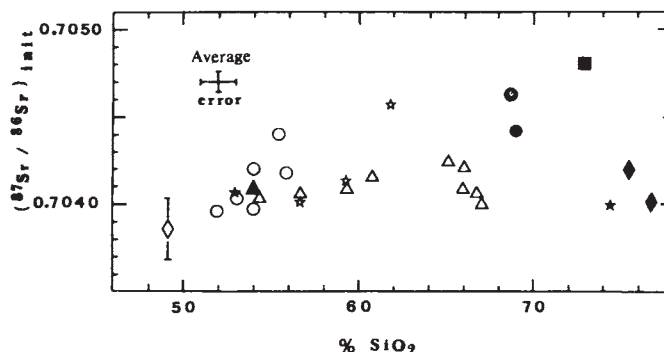


Fig. 2 A plot of initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio against SiO_2 (wt%). Filled and open symbols are used for pre- and post-caldera samples respectively. In stratigraphical sequence from oldest to youngest rocks, these are: Tpr ■ (CH42); Tpi ★ (HF15); Tprl ◆ (HF238 and HF239); Tppc ● (HF76); Tpb ▲ (CH24); Tpil ○ (HF2); Qbl ★ (CH4 to CH16); Qpc △ (CH68 to CH76); Qb2 ◇ (HF117); and Qb3 ○ (CH28 to CH47 except CH42).

The $^{87}\text{Sr}/^{86}\text{Sr}$ ratios for basalts, andesites and dacites from Los Humeros range from 0.7039 to 0.7046 and are comparable with similar rocks from other areas of the MVB¹⁴⁻¹⁶, Central America¹⁷⁻¹⁹, and other circum-Pacific active margins^{20,21}. The $^{87}\text{Sr}/^{86}\text{Sr}$ ratios for rhyodacites, rhyolites and ignimbrites from Los Humeros (0.7040–0.7048) are only very slightly higher than the associated more mafic rocks. Such low ratios for felsic volcanism have been reported only from Batopilas area²² of the Sierra Madre Occidental (SMO) in Mexico and from Nicaragua²³. However, most areas of the SMO and Central America show considerably higher initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios for rhyolitic and ignimbritic rocks²²⁻²⁵.

The $^{143}\text{Nd}/^{144}\text{Nd}$ ratios for the samples studied from Los Humeros caldera range from 0.51258 ± 2 to 0.51286 ± 3 (Tables 1 and 2). There is a good negative correlation ($r \approx -0.83$) between the initial $^{143}\text{Nd}/^{144}\text{Nd}$ and $^{87}\text{Sr}/^{86}\text{Sr}$ ratios for Los Humeros, similar to that observed for volcanic rocks from several areas²⁶⁻³⁰. The Nd and Sr isotope ratios are plotted in Fig. 3: for simplicity, only the fields defined by the data given in literature on mid-ocean ridge basalts (MORB), some oceanic islands and continental- and island-arcs are shown. Los Humeros data fall close to the 'mantle array' defined by MORB and oceanic island rocks. Further, their trend is almost parallel to that of the mantle array. These seem to be important observations bearing on the source of Los Humeros magmas and their evolution in the magma chamber.

In continental-arc environment several possible components must be considered for the origin of magma^{29,31,32}: (1) igneous rocks of the subducted oceanic crust; (2) sediments subducted with the downgoing slab; (3) mantle wedge beneath the arc; and (4) continental (lower, middle or upper) crust. The relative

Table 1 Analytical results on pre-caldera volcanism in Los Humeros

Sample	Rock-type*	$\text{SiO}_2^{\dagger}$ (wt%)	K_2O^* (wt%)	Rb (p.p.m.)	Sr (p.p.m.)	$(^{87}\text{Rb}/^{86}\text{Sr})^*$	$(^{87}\text{Sr}/^{86}\text{Sr})^{\ddagger}$	$(^{143}\text{Nd}/^{144}\text{Nd})^{\ddagger}$	$(^{87}\text{Sr}/^{86}\text{Sr})_{\text{initial}}^{\S}$
CH42	HK rhyolite	72.86	4.24	96.4 ± 0.9	104.8 ± 0.4	2.630 ± 27	0.70483 ± 4	0.51258 ± 2	0.70479
HF15	Aphyric rhyolitic pumice	74.37	3.07	133.3 ± 0.7	10.15 ± 0.04	37.600 ± 250	0.70451 ± 8	0.51272 ± 3	0.70398
HF238	HK pyroxene rhyolite	76.80	4.04	122.6 ± 0.7	29.0 ± 0.2	12.100 ± 110	0.70419 ± 9		0.70402
HF239	Aphyric rhyolite	75.55	2.95	87.0 ± 0.5	26.7 ± 0.1	9.330 ± 64	0.70434 ± 6		0.70421
HF76	HK rhyodacitic tuff	68.99	3.36	87.5 ± 0.5	108.9 ± 0.6	2.300 ± 18	0.70445 ± 4		0.70442
CH24	Basaltic andesite	54.02	1.27	30.2 ± 0.2	623 ± 2	0.139 ± 1	0.70409 ± 7		0.70409
HF2	Rhyodacitic ignimbrite	68.65	2.32	64.7 ± 0.4	160.7 ± 0.6	1.153 ± 8	0.70465 ± 4		0.70463

* The classification is based on K_2O - SiO_2 diagram⁴⁴ (HK = high-K); SiO_2 (wt%) by gravimetry¹⁰. The total propagated analytical errors range as follows: K_2O 0.5–1%; Rb 0.7–1.2% and Sr 0.3–0.5%. The samples are arranged in approximate sequence of decreasing age from top to bottom.

† The reported errors are total propagated errors multiplied by 10^3 .

‡ The $^{87}\text{Sr}/^{86}\text{Sr}$ ratios are normalized to $^{86}\text{Sr}/^{88}\text{Sr} = 0.11940$ and adjusted to SRM 987 $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.71021. The measured ratio for the SRM 987 standard is 0.71028_{-4}^{+0} (2σ , $n = 18$) during the period of measurement of about one year (1979–80). In this period, E & A SrCO_3 standard gave a value of 0.70807_{-3}^{+3} (2σ , $n = 9$). The $^{87}\text{Sr}/^{86}\text{Sr}$ ratios measured during 1981 are adjusted to E & A SrCO_3 $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.70800 when this standard gave a value of 0.70802_{-3}^{+3} (2σ , $n = 10$) after a resistor replacement in the machine. The $^{143}\text{Nd}/^{144}\text{Nd}$ ratios are normalized to $^{146}\text{Nd}/^{144}\text{Nd} = 0.72190$ and adjusted to BCR-1 $^{143}\text{Nd}/^{144}\text{Nd}$ ratio of 0.51265. The measured ratio for BCR-1 is 0.51256_{-3}^{+3} (2σ , $n = 6$). Further, the errors reported on individual $^{87}\text{Sr}/^{86}\text{Sr}$ and $^{143}\text{Nd}/^{144}\text{Nd}$ are two times the standard error of the mean ($2\sigma_{\text{E}}$) multiplied by 10^5 .

§ Initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios are obtained by correcting the measured $^{87}\text{Sr}/^{86}\text{Sr}$ ratios for the radiogenic ^{87}Sr growth during 1 Myr.

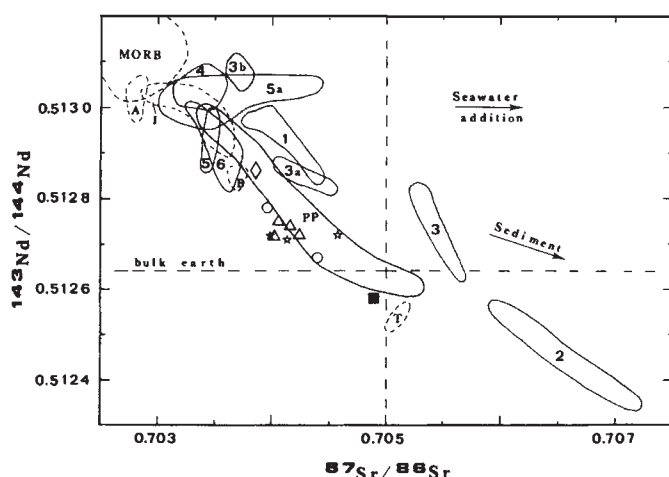


Fig. 3 A plot of $^{143}\text{Nd}/^{144}\text{Nd}$ versus $^{87}\text{Sr}/^{86}\text{Sr}$. The Nd and Sr isotope compositions have been adjusted to BCR-1 $^{143}\text{Nd}/^{144}\text{Nd} = 0.51265$ and E&A $^{87}\text{Sr}/^{86}\text{Sr} = 0.70800$ respectively. A, Ascension; I, Iceland; B, Bouvet; T, Tristan da Cunha; all oceanic island data from ref. 27; MORB^{26,27,45,46}. PP, Patagonian Plateau lavas²⁹. MORB, A, I, B, and T define the mantle array. Continental- and island-arc data: 1, Ecuador²⁹; 2, Chile²⁹; 3, Grenada⁴⁷; 3a, Dominicana⁴⁸; 3b, St Kitts⁴⁸ (all Lesser Antilles); 4, New Britain^{28,31}; 5, Esmeralda bank-Mariana arc⁴⁹; 5a, Mariana arc^{28,50}; 6, volcano arc³². Los Humeros data are plotted using the symbols as in Fig. 2.

importance of these components in the genesis of Los Humeros magmas can be assessed from the Nd-Sr isotope diagram (Fig. 3).

On interaction with seawater the $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of oceanic igneous rocks are modified towards higher values than their original unaltered composition^{27,33-35} but their $^{143}\text{Nd}/^{144}\text{Nd}$ ratios remain unmodified^{27,36}, because of the extremely low concentration of Nd in seawater as compared with that of Sr (refs 27, 37, 38). If material from the subducted oceanic crust (either directly by melting of the slab or indirectly as extracted aqueous fluids during its dehydration) contributes to the volcanic rocks of destructive margins, these rocks should show a displacement to high $^{87}\text{Sr}/^{86}\text{Sr}$ ratios at a given $^{143}\text{Nd}/^{144}\text{Nd}$ ratio (a shift towards the right of the mantle array) as is observed in many island- and continental-arc suites²⁹ (see Fig. 3). Authigenic sediments from the Pacific^{36,37,39} have $^{87}\text{Sr}/^{86}\text{Sr} \sim 0.709$ and $^{143}\text{Nd}/^{144}\text{Nd} \sim 0.5125$. Incorporation of such sediments would tend to modify the 'initial' mantle compositions

towards the general direction of the sediment vector drawn in Fig. 3. The actual path will, of course, depend on the Sr/Nd ratios of the two end-members⁴⁰. The Sr and Nd isotope compositions of detrital sediments in the Middle America trench are not precisely known but are expected to be different from the mantle sources. In fact, they may be similar to those of the continents³¹. Sediments do not seem to be subducted with the downgoing slab (Cocos plate) as the drilling on Leg 66 of the Deep Sea Drilling Project has shown a progressive underthrusting and uplift of trench deposits along the inner slope of the Middle America trench⁴¹.

If arc magmas are derived entirely from melting of the underlying mantle and there is no input from altered subducted slab or from continental crust, they should plot on the mantle array, as is the case with Los Humeros magmas. Large-scale contamination of the magmas with the continental crust is readily ruled out on the basis of rather narrow range observed in both the $^{87}\text{Sr}/^{86}\text{Sr}$ and $^{143}\text{Nd}/^{144}\text{Nd}$ ratios. The surprising uniformity of the Sr and Nd isotope ratios observed in post-caldera pumice deposits, in spite of the large range in their SiO_2 (54–67%) and trace element concentrations, constitutes a very strong argument in favour of very little, if any, interaction of 'post-caldera' magmas with the surrounding continental crust during their evolution in the magma chamber. It is only with the oldest rhyolite intrusion (sample CH42) during the pre-caldera evolution in the magma chamber that the possibility of some interaction with the continental crust could exist. The continental crust underlying the study area is likely to have much higher $^{87}\text{Sr}/^{86}\text{Sr}$ and lower $^{143}\text{Nd}/^{144}\text{Nd}$ ratios as compared with the 'bulk Earth' compositions. The isotopic compositions of the rhyolite intrusion could be generated by mixing a small proportion of such a continental crust will less-evolved magma if the Sr/Nd ratio of the crust is much smaller than that of the magma, thus resulting in a steep hyperbolic mixing curve^{40,42}.

Note that the field of Los Humeros volcanics overlaps with that of Patagonian plateau lavas of South America (Fig. 3). These basaltic lavas seem to have erupted in an extensional regime (similar to marginal basin in tectonic setting) behind the Andean cordillera²⁹. Based on fault mechanism of a large earthquake ($M = 7.3$) in a nearby area⁴³, it is possible that Los Humeros may also lie in a tensional regime. However, unlike Patagonia, an entire suite from basalt to rhyolite has erupted in Los Humeros and it is not clear whether the tensional regime in Los Humeros represents a marginal basin tectonic setting or is simply related to tensional stress associated with the downgoing slab.

I thank J.-G. Schilling for use of experimental facilities at the University of Rhode Island and R. Kingsley, P. Ogdan,

Table 2 Analytical results on post-caldera volcanism in Los Humeros

Sample	Rock-type*	SiO_2^* (wt%)	K_2O^* (wt%)	Rb (p.p.m.)	Sr (p.p.m.)	$^{87}\text{Rb}/^{86}\text{Sr}^+$	$^{87}\text{Sr}/^{86}\text{Sr}^\ddagger$	$^{143}\text{Nd}/^{144}\text{Nd}^\ddagger$
CH4	HK andesite	59.23	2.43	55.6 ± 0.5	482 ± 1.3	0.330 ± 3	0.70414 ± 6	0.51271 ± 4
CH7	HK andesite	61.74	2.93	73.4 ± 0.9	427 ± 1.5	0.492 ± 6	0.70457 ± 3	0.51272 ± 3
CH14	Andesite	56.55	1.79	40.5 ± 0.5	451 ± 1.3	0.257 ± 3	0.70401 ± 8	
CH16	Basaltic andesite	52.91	1.30	28.1 ± 0.2	437 ± 1.5	0.184 ± 1	0.70407 ± 7	
CH68	HK dacitic pumice	65.93	3.54	101 ± 1	226.8 ± 0.7	1.275 ± 13	0.70409 ± 7	
CH69	HK dacitic pumice	65.12	3.55	107.7 ± 0.7	262.2 ± 0.8	1.176 ± 8	0.70424 ± 5	0.51272 ± 3
CH70	HK dacitic pumice	66.74	3.87	102.4 ± 0.9	193.7 ± 0.7	1.513 ± 14	0.70406 ± 3	
CH71	Basaltic andesitic pumice	54.30	1.47	33.5 ± 0.2	491 ± 1.8	0.195 ± 1	0.70406 ± 7	0.51275 ± 2
CH72	HK rhyodacitic pumice	67.04	4.07	107 ± 1	164.1 ± 0.7	1.867 ± 19	0.70400 ± 6	0.51272 ± 4
CH73	HK andesitic pumice	56.58	2.32	58.9 ± 0.7	476 ± 1.3	0.354 ± 4	0.70406 ± 5	
CH74	HK andesitic pumice	60.80	2.46	65.6 ± 0.4	405 ± 1.3	0.464 ± 3	0.70416 ± 5	0.51274 ± 3
CH75	HK dacitic pumice	66.10	3.52	90.4 ± 0.9	245.3 ± 0.9	1.055 ± 11	0.70421 ± 2	
CH76	Andesitic pumice	59.33	2.08	52.3 ± 0.5	487 ± 1.3	0.307 ± 3	0.70409 ± 5	
HF117	Basalt	49.10	0.41	6.1 ± 0.1	367 ± 1.6	0.048 ± 1	0.70386 ± 18	0.51286 ± 3
CH28	Basaltic andesite	55.37	1.69	37.3 ± 0.3	541 ± 1.7	0.197 ± 2	0.70440 ± 7	0.51267 ± 3
CH31	Basaltic andesite	55.83	1.23	36.1 ± 0.3	540 ± 1.4	0.191 ± 2	0.70418 ± 5	
CH33	Basalt	51.86	1.23	24.5 ± 0.2	471 ± 1.2	0.149 ± 1	0.70396 ± 5	0.51278 ± 2
CH40	Basaltic andesite	53.05	1.43	29.1 ± 0.2	502 ± 1.3	0.166 ± 1	0.70403 ± 6	
CH46	Basaltic andesite	54.00	1.40	30.3 ± 0.2	441 ± 2	0.197 ± 2	0.70420 ± 8	
CH47	HK basaltic andesite	53.97	2.04	41.1 ± 1.3	456 ± 2	0.258 ± 9	0.70397 ± 6	

Relatively poor $^{87}\text{Sr}/^{86}\text{Sr}$ measurement for HF117 may be due to the presence of high Ca in the Sr-fraction and the resulting instability of the ion beam.

*, † and ‡, See legend to Table 1.

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Overlapping spreading centres: new accretion geometry on the East Pacific Rise

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In a detailed Seabeam investigation of the East Pacific Rise (EPR) from 8°N to 18°N, a new kind of volcano-tectonic geometry associated with fast-spreading centres has been discovered (Figs 1, 2). At several locations along the rise axis the neovolcanic zone is discontinuous, and is laterally offset a short distance (1–15 km). In contrast to a classic ridge-transform-ridge plate boundary, however, the offset ridge terminations overlap each other by a distance approximately equal to or greater than the offset. They curve sharply towards each other and often merge into one another along strike. Separating the overlapping spreading centres (OSCs) is a closed contour depression up to several hundred metres deep which is sub-parallel to the trend of the OSCs. The region between the OSCs is a complex zone of both shear and rotational deformation with no obvious transform parallel structures. Based on wax model studies of spreading centres, we suggest here that transform faults fail to develop at fast spreading centres where the lateral offsets are small (<15 km), because the lithosphere is too thin and weak to maintain a classic, rigid plate spreading centre-transform fault pattern. The OSC geometry is unstable and evolves rapidly. One of the two OSCs prevails while the other is abandoned. A significant area of sea floor created at fast spreading rates may bear the imprint of this newly observed process.

In July and August 1982 we conducted an extensive seabeam study of the EPR with nearly continuous coverage of every spreading segment and transform fault between 8°20' N and 18°30' N. The seabeam system aboard RV *Thomas Washington* insonifies the ocean floor with an array of 16 beams, each with a width of 2°40' (ref. 1), and the total width of the beam swath is approximately two-thirds of the water depth. This allows

collection of continuous or overlapping topographic information at high resolution over large areas of the sea floor. During our survey one swath completely insonified the rise axis neovolcanic zone, allowing continuous along-strike coverage of this zone over a 1,200-km length of rise crest. For part of the cruise we used the new Navstar global positioning system (GPS), and while transit satellites offer 250–500 m accuracy after smoothing of the data, GPS provides an estimated accuracy of 20 m or better with fixes every 2 s during the times available.

Along the 1,200-km long segment of fast accreting plate boundary investigated in our study, the total spreading rate varies from 9 cm yr⁻¹ at 18°N to 12 cm yr⁻¹ at 8°N (ref. 2). The axis of accretion (neovolcanic zone) is a linear zone which is only 1.0–2.0 km wide and is relatively continuous along strike, in keeping with other studies of the EPR^{3–5}. The continuous along strike perspective demonstrates, however, that the volcano-tectonic character of the rise axis and the depth to the rise crest are much more variable than earlier investigations have suggested. Over distances of 20–40 km the morphology of the neovolcanic zone evolves from a 1–2 km wide peaked ridge into a rifted swell with a 5–50 m deep summit graben, and the depth to the axis varies smoothly between 2,500 and 2,800 m. These morphological and bathymetric changes are clearly developed near the three major transform faults we surveyed (Fig. 1; Siquieros, Clipperton, Orozco) and, surprisingly, are also associated with a ridge axis geometry that has to date escaped recognition. The nature of this new mid-ocean ridge geometry is the focus of this discussion.

At several locations along the rise axis (Fig. 1) we define an accretion geometry characterized by two overlapping neovolcanic zones that curve towards each other (Fig. 2) enclosing an elongate depression (Fig. 2). We suggest that the term 'overlapping spreading centres' be used to describe this kind of tectonic regime. The dimensions of the OSCs are variable. At 9°N and 11°45'N, the largest systems identified, the neovolcanic zones are offset 8–15 km, the ridge axes overlap each other by 25–35 km and the relief of the enclosed basins exceeds 500 m. At other localities the dimensions of the OSCs are smaller with offsets as small as 1–2 km. Along strike as the OSCs are approached, the depth of the rise axis increases by 50 m to 300 m, the width of the neovolcanic zone decreases continuously, and the number of conical volcanic centres diminishes markedly. The along-strike change in morphology suggests a decrease in volcanic budget as the OSCs are approached. The intervening deep hole sandwiched between

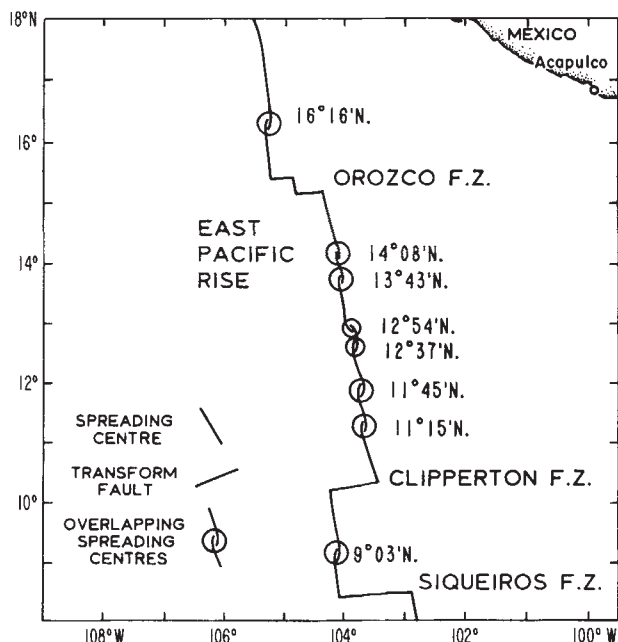


Fig. 1 The Seabeam survey covered the entire East Pacific Rise including transform faults from 8°20' N to 18°30' N. Circled areas show where overlapping spreading centres were mapped.

the OSCs is elliptical in shape with the long axis sub-parallel to the two bounding spreading centres, and the closed basin is devoid of structures with transform fault orientations (Fig. 2).

Notably, eight OSCs have been mapped along a 1,200 km segment of the EPR where none had been discovered before. Similar structures also appear to be common on the fast spreading southern EPR^{6,7,19}. However, none have been detected on the slow-spreading Mid-Atlantic Ridge, even where there is considerable multi-beam bathymetric coverage^{8,9}. Perhaps the development of OSCs is related to spreading rate. The lithosphere in the vicinity of the OSCs is likely to be thin, as little as 2–3 km if an axial magma chamber is present^{10–12}. If small offsets of the axial neovolcanic zone develop, then the offset pieces of lithosphere may be too thin and weak to maintain a rigid plate ridge–transform–ridge geometry. In such conditions, the lithosphere between the offset ridges may experience pervasive shear strain and non-rigid plate deformation.

We have found that a striking resemblance to the OSC geometry develops in wax model experiments of spreading centres. In these experiments, mid-ocean ridge processes are simulated by spreading paraffin plates atop hot molten wax. In the original experiments, Oldenburg and Brune¹³ found that orthogonal spreading centre–transform fault patterns were stable for slow opening rates of the wax plates ($< 15 \text{ mm s}^{-1}$), but were unstable at fast opening rates. We have recreated the wax experiment to investigate in detail what occurs at fast spreading rates for small ridge offsets in the model. We initiate the experiment in the same way as Oldenburg and Brune with two sub-parallel knife cuts in the frozen wax surface (Fig. 3a), but increasing the spreading rate to $> 20 \text{ mm s}^{-1}$. As the wax plate is pulled in a direction orthogonal to the spreading centres (knife cuts), the spreading centres propagate along strike (Fig. 3b). They continue to propagate until they overlap each other in an *en echelon* sense (Fig. 3c). This overlapping geometry results in non-rigid plate behaviour, as the overlap zone is subjected to both progressive shear and rotational deformation. We have observed rotations of 5°–30° in the overlap zone, rotations of 10°–15° being typical. The tips of the OSCs are sheared towards each other in this process. As spreading proceeds, the OSCs continue to propagate along strike and to curve towards each other until one OSC tip links with the other OSC (Fig. 3d). A continuous spreading centre is established as one OSC prevails and the other becomes inactive. The abandoned OSC and the overlap zone are spread away to one side as spreading continues along the prevailing OSC (Fig. 3e).

The experiment was run again with a large ($> 2 \text{ cm}$) lateral separation between the two initial spreading centres (knife cuts). In these conditions, a stable rigid plate transform fault developed at $\sim 90^\circ$ to the spreading centre, even at fast opening rates.

The scenario indicated in our wax model studies may be a reasonable guide to the behaviour of the fast spreading EPR and to the development and evolution of OSCs in the Pacific. The various evolutionary phases of OSCs depicted in Fig. 3c–e are clearly displayed in the morphology of the eight OSCs charted. For example, the OSCs in Fig. 2 correspond to Fig. 3c, d, the OSCs at 13°43' N to Fig. 3d and the OSCs at 16°16' N to Fig. 3e. ANGUS photographic coverage was obtained over an OSC pair on the EPR at 19° S and it was found that one OSC was active while the other was mantled by sediment and apparently inactive, corresponding to the evolutionary phase in Fig. 3e (ref. 7).

Based on the wax model studies, we suggest that OSCs represent a rapidly evolving expression of how oceanic lithosphere is accreted at fast spreading rates. The liberation of large volumes of basaltic melt from asthenospheric sources and the emplacement of magma in shallow-level chambers beneath the ridge axis is a discontinuous process resulting in episodic swelling of the steady-state axial magma chamber and episodic extrusion of basalts on the sea floor^{14–16}. Viewed along strike, different portions of the rise axis are likely to be active at any given time while intervening regions will be dormant¹⁷. As crustal extension continues with spreading, a phase of localized magma replenishment occurs and eventually feeder dykes break through to the surface feeding lava flows. We suggest that volcanism and crustal growth in the neovolcanic zone will migrate away from such volcanic centres, propagating in a direction parallel to the strike of the rise as extensional processes continue.

The initiation of volcanism might correspond to the initial knife cuts made in the wax plate in Fig. 3a, and the OSC

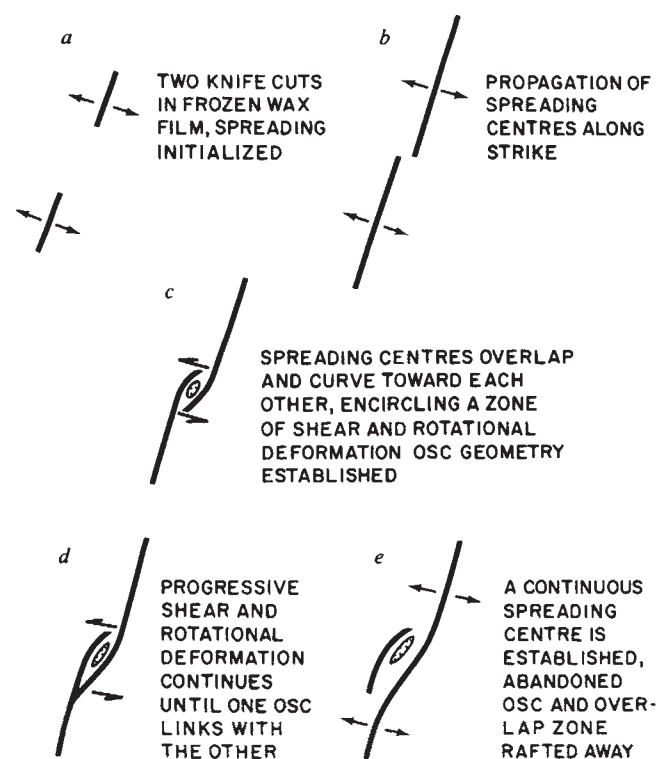


Fig. 2 Two examples of overlapping spreading centres. The OSCs at 9°N are typical of the larger offset case (5–15 km), while the 12°54' N area is typical of the small offset case (1–5 km). The spreading centres overlap by a distance which is equal to or greater than their lateral offset. A deep hole separates the OSCs. At 9°N the west OSC bends west before curving to the east, and may be in the process of being abandoned as outlined in Fig. 3.

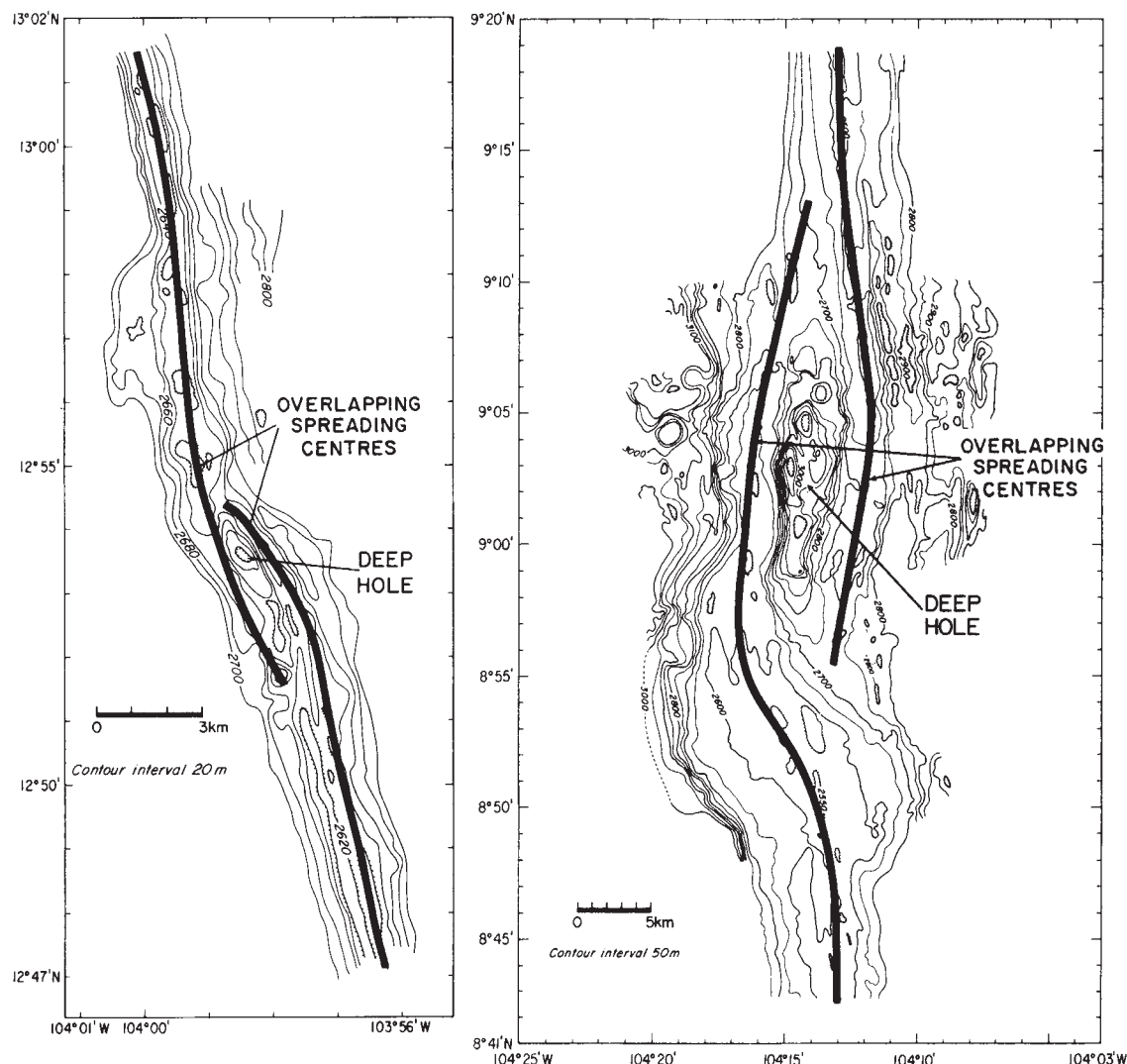


Fig. 3 Sketches of the evolution of overlapping spreading centres observed in wax model studies. The experiment is initiated with two knife cuts in the frozen wax surface, and then the wax film is pulled apart in a direction perpendicular to the trend of the cuts (*a*). The spreading centres propagate along strike as spreading proceeds (*b*). At fast opening rates a stable orthogonal transform fault does not develop and the spreading centres overlap each other. The tips of the OSCs are sheared towards each other, encircling a zone of complex shear and rotational deformation (*c*). Progressive shear and rotational deformation continue in the overlap zone until one OSC tip links with the other OSC (*d*). The abandoned OSC and overlap zone are rafted away as the prevailing OSC becomes the single through-going spreading centre. The resulting spreading centre is slightly sinuous and OSCs may recur near this bend. The eight OSCs charted between 8°N and 18°N on the EPR all resemble one of the evolutionary phases produced in the above wax model.

morphology may represent what happens when two neovolcanic zones migrate towards each other, but fail to meet head-on. This may occur because of either heterogeneities in the lithosphere or a slight sinuosity in the trend of the spreading centre. As the tips of accretion approach and begin to overlap, a zone of shear develops between them. A classic transform fault does not develop because the offset is small and, at fast spreading rates, the lithosphere is too young, thin and weak to support a rigid plate transform geometry. The spreading centres are sheared towards each other and continue to propagate along strike, creating a curving spreading centre overlap characteristic of the OSC geometry. The overlapping configuration that we see displayed on the sea floor must be unstable. In a relatively short time one of the overlapping segments will cut through the young and thin lithosphere, linking up with the other limb (Fig. 3*d*). This will isolate a short, curved, relict spreading segment. The morphological product of this evolutionary phase of accretion will be a gentle sinuous bend in the axis of the rise that is bordered by an anomalously deep trough and by an arcuate rise concave towards the spreading centre (Fig. 3*e*). Our along-strike survey focused on the neovolcanic zone and it is interesting that in plan view, 30–40 km long segments of the rise axis create a sinuous pattern. Given the large overlap of the OSCs (up to 35 km) a significant amount of sea floor

relief in the ocean basins may owe its origin to this process, and relict OSCs would appear in older lithosphere as ridges and troughs with oblique trends relative to the regional fabric. Repeated regeneration of OSCs along a sinuous rise may create topography similar to fracture zone traces.

Several questions remain unanswered regarding this new discovery. Do OSCs occur only at intermediate- to fast-spreading rates and only for small lateral displacements of the spreading axis? If the strength and thickness of the lithosphere are important factors, then an important constraint is that the largest lateral separation between OSCs is ~15–20 km, corresponding to an age for the affected lithosphere of ~0.25 Myr. Perhaps lithosphere older than 0.25 Myr is sufficiently thick and strong to impede non-rigid deformation.

What is the origin of the deep hole between the OSCs? Our model suggests that it is a piece of sheared and rotated lithosphere, but that does not explain the depression. Perhaps it is an extensional basin associated with a short-lived shear couple generated during the evolution of the OSC geometry. Alternatively, it may be a volcanic collapse feature associated with an underlying magma chamber. What is the relationship between the OSCs and the axial magma chamber? The OSC separations are small enough (1–15 km) for the entire OSC zone to be underlain by a magma chamber^{10–12}. If so, the OSC geometry

may be peculiar to the thin brittle lid overlying the axial magma chamber. OSC offsets may even be limited by the width of the axial magma chamber. Seismic refraction studies are needed to test these ideas.

Can magnetic anomalies be analysed to test the model for the evolution of OSCs we have proposed? The rotation in the overlap zone predicted by the wax model should have a substantial magnetic signal due to rotation of the magnetic vector. In fact, large negative magnetic anomalies are recorded over the deep holes where the anomalies should be positive. The anomaly is too large to be accounted for by topography and may indicate rotation. Three-dimensional inversion studies of the magnetic field are underway. How are the petrology and geochemistry of the oceanic crust affected? We have measured large positive magnetic anomalies near the tips of the OSCs, a

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possible indication of the eruption of high Fe–Ti basalts in these areas. Magnetic modelling is in progress and careful rock sampling is needed to test this hypothesis.

Finally, there is a similarity between our model for OSC development and the propagating rift hypothesis, in that both hypotheses involve the along-strike propagation of spreading centre activity¹⁸. Propagating rifts, which appear to be common at fast but not at slow spreading centres, occur in response to changes in spreading direction. If there is a regional reorientation of stresses and a need for the spreading direction to change, then OSCs might become nucleation points for large scale rift propagation.

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Symbiotic chemoautotrophic bacteria in marine invertebrates from sulphide-rich habitats

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The primary base of the food chain of the dense animal populations found clustered around deep-sea hydrothermal vents^{1,2} appears to be chemoautotrophic bacteria, whose energy source is geothermally reduced hydrogen sulphide emitted from the vents^{3,4}. Recently, a symbiotic association has been postulated⁵ between chemoautotrophic sulphur-oxidizing bacteria and the vent tubeworm, *Riftia pachyptila* Jones (phylum Pogonophora) on the basis of histological⁵ and enzymatic⁶ evidence. Hydrothermal vents thus appear to be a spectacular example of the role of reduced inorganic elements in animal nutrition. Marine muds and salt marsh sediments also produce a continuous supply of reduced sulphur compounds^{7,8}, so the possibility arises that they support similar symbiotic associations^{5,9}. I now present microscopic, enzymatic, and physiological evidence for the occurrence of intracellular sulphur-oxidizing chemoautotrophic bacteria in a bivalve, *Solemya velum* Say (phylum Mollusca), found in reducing muds of eelgrass beds. Bacterial symbionts were also observed in other animals from a variety of sulphide-rich habitats. It thus seems that symbiotic relationships with bacteria are widespread among sulphide-habitat marine invertebrates, and may have a significant role in their nutrition.

Two Atlantic coast bivalves were analysed for the presence and activity of chemoautotrophic bacteria by: (1) assaying for ribulose-1,5-bisphosphate carboxylase (RuBPCase) activity; (2) transmission electron microscopy; (3) epifluorescent microscopy; (4) assays of lipopolysaccharide content; and (5) sulphide and thiosulphate enhancement of carbon dioxide fixation. *S. velum*, a protobranch bivalve¹⁰, collected from eelgrass beds near Woods Hole, was compared with *Geukensia demissa* (Dillwyn), the ribbed marsh mussel^{10,11}, obtained from creek banks in Little Sippewissett Salt Marsh, Falmouth,

Massachusetts. These two bivalves both have access to the inorganic compounds necessary for sulphur-based chemoautotrophic metabolism: reduced inorganic sulphur compounds from the reducing sediments in which they live, oxygen from the overlying seawater, and CO₂ from both sources.

RuBPCase activity is a good indicator of the presence of chemoautotrophic symbionts, as it is only found in autotrophic organisms¹² (in which it is the major CO₂ fixation enzyme). RuBPCase activity was detected only in the gill tissue of *S. velum* (Fig. 1). The absence of RuBPCase activity in *G. demissa* gill tissue showed that the activity measured in *S. velum* was not due to contamination by phytoplankton or free-living chemoautotrophic bacteria (Fig. 1).

Transmission electron microscopy (TEM) of thin sections of *S. velum* gill tissue revealed subcellular inclusions with characteristics of prokaryotic cells, including: (1) cell morphology and size; (2) the absence of any internal membrane bound organelles; (3) the presence of a non-membrane-bound nuclear region; and (4) a cell envelope which resembles that of Gram-negative bacteria (Fig. 2). Gill tissue homogenates of *S. velum* stained with acridine orange, a nucleic acid specific stain, and examined by epifluorescent microscopy¹³ demonstrated the presence of uniformly fluorescent rod-shaped cells resembling in size and shape the bacteria observed by TEM. Similar cell inclusions were not seen in thin sections or in tissue homogenates of *G. demissa* gill tissue.

Lipopolysaccharide, diagnostic of the outer cell wall of Gram-negative bacteria¹⁴, was found to be 1,000 times more abundant (2 µg per g wet weight) in *S. velum* gills than in *G. demissa*, indicating the presence of a population of Gram-negative bacteria far in excess of that which could be attributable to surface bacterial contamination.

The bacteria observed in *S. velum* gill tissue are rod-shaped unicells of length ranging from 2 to 10 µm (mean ± s.d. 1.1 ± 0.2 µm × 4.4 ± 0.9 µm; N = 30, determined by phase contrast microscopy). Direct counts of rod-shaped fluorescent cells indicated that there were 1.2 ± 0.4 × 10⁶ cells per g wet weight (mean ± s.d.; n = 4). These bacteria appear intracellular, and are enclosed by an outer membrane, presumably that of the host animal cell (Fig. 2). This structural organization is reminiscent of symbiotic systems in root nodules involving nitrogen-fixing bacteria of the genus *Rhizobium*¹⁵.

The incorporation of ¹⁴CO₂ in gill tissue in the presence of sulphide and thiosulphate was examined to test whether

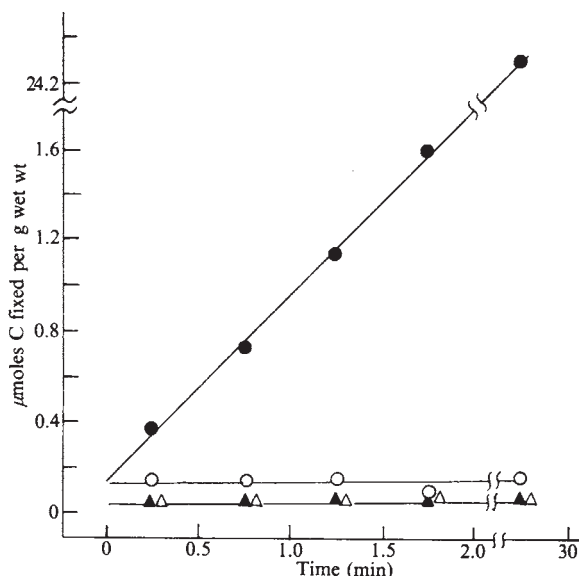


Fig. 1 Time course of RuBP-dependent CO_2 fixation in cell-free extracts of the gill tissues of *S. velum* and *G. demissa*. (●) *S. velum* gill extracts with RuBP added; the fixation was linear ($r^2 = 0.99$) for over 30 min, and the rate (equivalent to $0.81 \mu\text{mol fixed CO}_2$ per g wet weight per min) was at least two orders of magnitude more than any background fixation taking place in the absence of RuBP (○). No RuBP-dependent CO_2 fixation could be measured in extracts of *G. demissa* gill tissue (▲) above the control (△), nor was activity detected in cell-free extracts of the mantle or foot tissue of either mollusc (data not shown).

Methods: RuBCase activity was determined by comparing the fixation of $\text{NaH}^{14}\text{CO}_3$ in the presence and absence of RuBP¹⁷. Animals were collected within 24 h of the assay, kept in mud from the collection sites with running seawater, and placed in filtered ($0.22 \mu\text{m}$) seawater 1 h before dissection. Cell-free extracts were obtained by sonification ($4 \times 15 \text{ s}$) of tissue (gill, mantle, foot) homogenates in the assay buffer (w/v 1:4) and centrifugation for 30 min at $27,000g$ (4°C). Sonification increased RuBPCase activity threefold in extracts of *S. velum* gill tissue in a preliminary experiment (unpublished data). Subsequently all tissue homogenates were sonified before testing for RuBPCase activity. Small pieces of hypobranchial gland remained attached to *S. velum* gill tissue even with careful dissection. Bacteria were not observed in preliminary TEM examination of *S. velum* hypobranchial gland. Eel grass sediments smelled strongly of hydrogen sulphide. In the pore water of Sippewissett Marsh sulphide concentrations ranged from 0.1 to 0.6 mM, thiosulphate concentrations from 0.1 to 0.5 mM (annual mean values for different sites)²⁵.

reduced sulphur compounds can serve as an energy source for carbon fixation (Table 1). The addition of either reduced sulphur compound greatly enhanced $^{14}\text{CO}_2$ incorporation in *S. velum* gills, while little to no enhancement was observed in *G. demissa* tissue.

I conclude that the rod-shaped prokaryotic cells observed in *S. velum* are symbiotic bacteria capable of CO_2 fixation, and that carbon fixation is enhanced by reduced sulphur compounds. The RuBPCase activity per mg bacterial protein in *S. velum* (calculated from cell volume and conversion factors¹⁶) is comparable with that measured in *Thiobacillus neopolitanus*, an obligate chemoautotrophic sulphur bacterium¹⁷. Work is in progress to isolate and characterize the symbiotic bacteria from *S. velum* and to assess the role of these symbionts in the carbon metabolism of the animal.

A TEM survey of other invertebrates from sulphide-rich habitats revealed bacteria (inclusions resembling prokaryotic cells on the basis of the characteristics listed above) in the gill tissue of bivalves and in the trophosome tissue of vestimentiferans (Fig. 3). Although the bacteria differed in size and

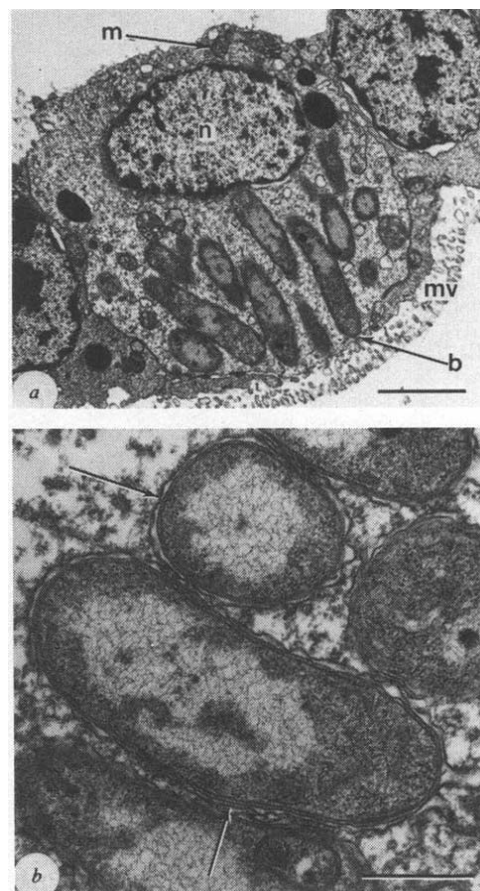


Fig. 2 Transmission electron micrographs showing bacteria in gill tissue cells of *S. velum*. **a**, Transverse section of gill filament showing bacteria within an animal cell. Animal nuclei are found in the basal region of the cells and bacteria are typically observed in the apical region of cells. Scale bar, $3 \mu\text{m}$. **b**, Bacteria; n, animal cell nucleus; m, mitochondria; mv, microvilli on gill filament surface. **b**, Higher magnification showing oblique and transverse sections of rod-shaped bacteria. The Gram-negative bacteria are surrounded by an outer unit membrane (arrows), possibly that of the host cell. Scale bar, $0.5 \mu\text{m}$. Tissue was fixed in 3% glutaraldehyde in marine (veronal acetate) buffer²⁶, post-fixed in OsO_4 , embedded in Spurr's resin and sections stained with uranyl acetate and lead citrate.

Table 1 Rates of CO_2 fixation in gill tissue of *S. velum* and *G. demissa*

Species	Sulphur compound	+ Sulphur	- Sulphur
<i>S. velum</i>	0.2 mM Na_2S	4.5	0.70
		4.6	0.49
	1.0 mM $\text{Na}_2\text{S}_2\text{O}_3$	6.5	0.57
		1.6*	0.56
		8.3	0.43
<i>G. demissa</i>	0.2 mM Na_2S	9.1	0.35
		0.13	0.08
	1.0 mM $\text{Na}_2\text{S}_2\text{O}_3$	0.18	0.09
		0.10	0.08
		0.10	0.09

Data are expressed as $\mu\text{moles CO}_2$ fixed per g wet weight per h. Whole gills (*S. velum*), or pieces of gill (*G. demissa*), were incubated in filtered ($0.22 \mu\text{m}$) seawater (pH 8.1, 22°C) containing $4 \mu\text{Ci ml}^{-1}$ $\text{NaH}^{14}\text{CO}_3$ (53 mCi mmol^{-1}). At the end of the incubation period (3 h) the gills were transferred to acidified filtered seawater (pH 2.5) for 1 h to remove inorganic carbonate. Tissues were solubilized and radioactivity determined. Background counts, amounting to less than 2% of the lowest tissue sample value, were subtracted from all sample counts. Counting efficiency was determined by the channels ratio method and with internal standards, which agreed.

* The gill tissue of this animal was noted during dissection to be a pale, sickly colour (in contrast to the deep red of other specimens of *S. velum*).

Fig. 3 Transmission electron micrographs showing bacteria in the trophosome tissue of vestimentiferans (phylum Pogonophora) (a, b) and in the gills of bivalves (phylum Mollusca) (c–f). Animals were collected from sulphide-rich deep-sea hydrothermal rift vents (a, c, f), fault vents (b, d), and reducing sediments (e). a, *Riftia pachyptila* Jones (collection site 21° N, East Pacific Rise). Rod-shaped and large pleomorphic bacteria have been observed in addition to predominantly coccoid-shaped cells as seen in specimens examined previously⁵. The additional membrane surrounding the bacterial cells was not obvious in prior TEM examination of *R. pachyptila* trophosome tissue⁵, probably due to poor fixation (tissue available previously for electron microscopy had been fixed in 5% formalin in seawater). Scale bar, 1 µm. b, Vestimentiferan (San Diego Trough), unnamed new species of the family Lamelli-brachidae²⁷ (M. Jones, personal communication). Membrane-bound vesicles were observed in the bacteria in both species of vestimentiferans. Scale bar, 1 µm. c, *Calyptogenia magnifica* Boss and Turner (21° N site), the giant white clam found at the deep-sea hydrothermal vents²³. Similar coccoid-shaped bacteria were also observed in the gill tissue of clams collected at the Galapagos Rift vent, but the fixation (5% formalin in seawater) was of poor

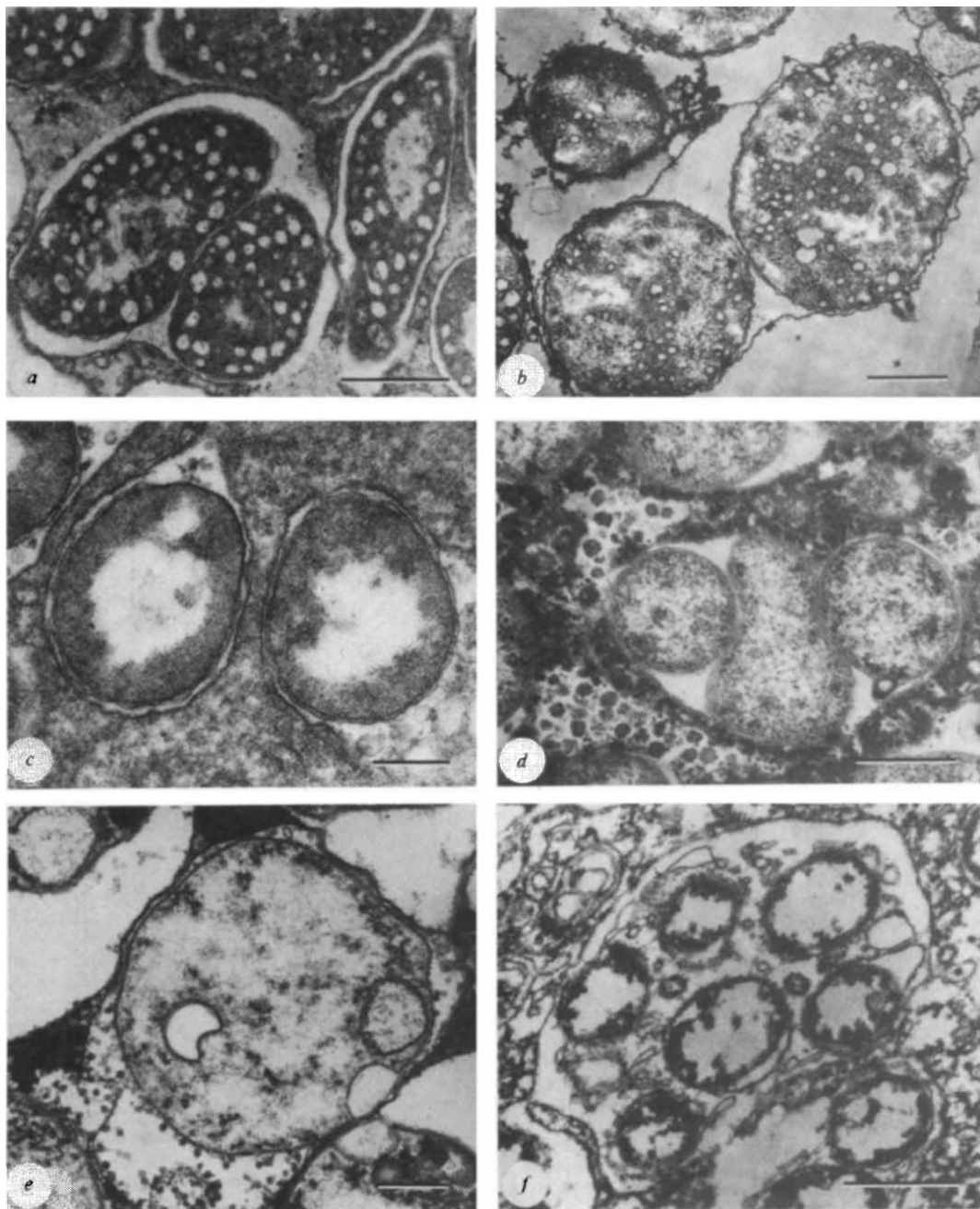
quality for electron microscopy. Scale bar, 0.25 µm. d, *C. pacifica* Dall (San Diego Trough). Glycogen (rosette-like structures seen in TEM) was observed in animal cell cytoplasm outside packets of pleomorphic-shaped bacteria. Scale bar, 0.5 µm. e, *Lucinoma annulata* Reeve (Santa Barbara Basin). Rod-shaped as well as coccoid-shaped bacteria (shown here) were observed in thin sections. Scale bar, 0.5 µm. f, unnamed mytilid (Galapagos Rift). The only vent mussel tissue available for electron microscopy was fixed in 5% formalin in seawater. Although the fixation for electron microscopy is very poor, the similarity of organization of these membrane-bound inclusions to that of the bacteria observed in the RuBPCase-containing gill tissues of the other bivalves examined here suggests that symbiotic bacteria also occur in this vent mussel. Scale bar, 0.5 µm.

Methods: Tissues were fixed in 3% glutaraldehyde in marine buffer²⁶ (b, d, e), 0.1 M cacodylate buffer with 0.4 M NaCl (a, c) or in 5% formalin in seawater (f), postfixed in OsO₄, embedded, and sections stained with lead citrate and uranyl acetate. Sulphide concentrations of up to 160 µM have been measured in mixed vent and ambient seawater at the deep-sea hydrothermal vents⁴. Although chemical measurements have not been made at the San Diego Trough, an area of suspected hydrothermal venting²⁸, the sediments brought up with the animals on DSRV *Sea Cliff* smelled strongly of hydrogen sulphide. High sulphide concentrations have been reported in the Santa Barbara basin²⁹.

ultrastructure among these animals, certain characteristics were shared with those observed in *S. velum*. In all animals examined, the bacteria appeared to have typical Gram-negative cell walls, and to be enclosed by an outer membrane, either individually or in groups of two or more (Fig. 3). In the bivalves, as in *S. velum* (Fig. 2a), the bacteria in the gills appeared to be intracellular. Intracellular bacteria have also been observed in the trophosome tissue of both species of vestimentiferan,

however it is not yet certain that all trophosome bacteria are contained within animal cells. Furthermore, as shown here for *S. velum*, RuBPCase activity has been detected in these bacteria-containing tissues¹⁸.

These TEM observations are critical evidence that the enzyme activities measured by Felbeck *et al.*¹⁸ reflect internal chemoautotrophic symbionts, rather than external contaminants or animal chemoautotrophy⁶. Although all the



animals studied here live in sulphide-rich habitats, it is not yet known whether sulphide or other reduced inorganic compounds serve as the energy source for the chemoautotrophic symbionts. Similar symbioses may also occur in several other small pogonophorans collected from sediments of the continental slope and deep fjords¹⁹, and in a reducing sediment oligochaete, *Phallodrilus leukodermatus* Giere²⁰.

In addition to their occurrence in sulphide-rich habitats, these marine invertebrates having the putative symbionts (this study and refs 5, 19, 18, 20) share certain striking morphological characteristics. Pogonophorans^{19,21} and the oligochaete *P. leukodermatus*²⁰ lack mouth and gut. Bivalves of the genera *Solemya*²² and *Calyplogena*²³, of the family Lucinidae²⁴, and the recently discovered vent mytilid (V. Kenk, personal communication) all have very small guts and feeding appendages, and thick, fleshy gills. On the other hand, *G. demissa* has a typical mytilid digestive tract, and pale thin gills that do not contain symbiotic chemoautotrophs. This survey strongly suggests that the invertebrates that form such symbiotic relationships with bacteria have adapted morphologically to internal chemoautotrophic CO₂ fixation (and possibly uptake of dissolved organic compounds^{19,22}) as their major source of nutrition.

The findings presented here, and those of others^{5,19,18,20}, suggest that symbiotic chemoautotrophic bacteria occur in many marine invertebrates inhabiting sulphide-rich or reducing habitats, and that the bacteria are important to: (1) the nutrition and notably the energy source of these organisms, (2) the animals' distribution, and (3) the overall productivity of these communities.

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First observation of a muscle spindle in fish

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In many groups of vertebrates, the muscle spindle is a specialized sensory organ for the detection of muscle stretching. The structure of the spindle varies among vertebrate classes^{1–3}. Moreover, Barker² has asserted that Amphibia are the most primitive vertebrates to possess muscle spindles. Extensive studies, made mainly on the locomotor myotome^{4–11}, seem to show that the muscle receptors of fish are less specialized than those of more advanced animals, and that muscle spindles are absent. However, little attention has been paid to the jaw-closing muscle. We report here our finding of a very simple muscle spindle with a single intrafusal fibre in the well-developed jaw-closing muscle, adductor mandibulae, in a primitive teleostean, *Oncorhynchus masou* (Brevoort).

Adult landlocked Japanese salmon, *O. masou*, of about 190 mm total length, were maintained in tanks of circulating tap water at 15 °C. Ecological and ethological characteristics of this species have been observed by us in the field and aquarium (90 × 45 × 45 cm). Five fish were stunned by a blow to the head and killed by decapitation. The posterior part of the a. mandibulae (Fig. 1a) was dissected from the fish and examined under light microscopy.

The a. mandibulae muscle was divided into a superficial portion, composed of muscle fibres of relatively large diameter, and a profundus portion. Muscle spindles were observed among the superficial extrafusal fibres. Figure 1b and c are low- and high-power photographs of a typical muscle spindle found in the a. mandibulae. In the muscle spindle, a single intrafusal fibre was observed in the capsules. The diameter of the extrafusal fibres in the superficial portion was 8.4–41.9 µm, while the diameter of the intrafusal fibre was about 2.8 µm at the polar region. Thus, fibres of small diameter were not observed among the extrafusal fibres. The length of the muscle spindle was about 1,800 µm in the serial sections. Figure 1d shows the primary nerve ending of a muscle spindle in which an afferent nerve fibre makes a large spiral course around the intrafusal fibre. Only one or two muscle spindles were observed in the a. mandibulae muscle.

Ecological and ethological observations have shown that *O. masou* feed chiefly on aquatic insect larvae carried by the current or on small fish of other species occurring in rapids where the water is swift and turbulent, in which case they usually show swift feeding activity. Moreover, they display a chewing-like movement of the jaw to manipulate comparatively large prey for deglutition, whereas, if the prey is small, it is swallowed rapidly. Furthermore, they often fight each other, using their small teeth to bite vigorously one another's fins and other body parts and keep biting for a short time.

In general, a muscle spindle is composed of a bundle of small intrafusal fibres receiving both motor and sensor innervation. In the Reptilia, the number of intrafusal fibres in the muscle spindles show species differences^{3,12,13}, while in Amphibia, thought to be the most primitive vertebrate classes to possess muscle spindles, muscle spindles with several intrafusal fibres have been reported¹⁴. However, Maeda¹⁵ has reported that the histochemical properties of the a. mandibulae in *O. masou* are more similar to those of the masseter muscle in mammals than to those of the locomotor myotome in fish. These similarities suggest the functional complexity of the muscle in comparison with that of other fish muscles; this is also suggested from observations of feeding activity and social behaviour in the field

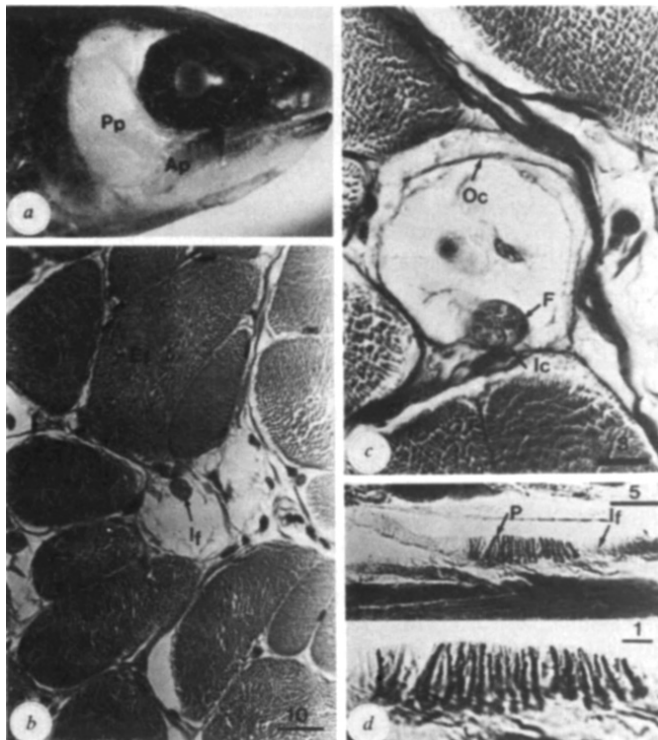


Fig. 1 a, Adductor mandibulae muscle of *Onchorhynchus masou* ($\times 1$). b, c, Transverse sections of muscle spindles (b, haematoxylin and eosin staining; c, Azan staining; d, protein silver staining). The posterior part of the a. mandibulae was dissected from the fish and fixed in Bouin's fluid. Sections were embedded in paraffin and cut longitudinally or transversely at $10\text{ }\mu\text{m}$. Transverse sections were stained with haematoxylin and eosin or with azan, whereas longitudinal sections were stained with protein silver. A Nikon light microscope (model UFX) was used to observe the specimens. The diameter of muscle fibres was measured by Canon Desigram (BX1). Pp, posterior part; Ap, anterior part; Ef, extrafusal fibre; If, intrafusal fibre; F, fibrocyte; Ic, inner capsule; Oc, outer capsule; P, primary nerve ending. In b, c and d the bars and numbers above them indicate the dimensions (μm).

and aquarium. On the other hand, Kubota^{16,17} asserted that many muscle spindles exist in the jaw-closing muscle, although there are few in the jaw-opening muscle. He further claimed that there is an important relationship between differences in food habits and the distribution of muscle spindles in masticatory muscles. Therefore, muscle spindles in the superficial portion of a. mandibulae may also have a key role in the subtle control of closing the jaw in *O. masou*.

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Presence in brain of synenkephalin, a proenkephalin-immunoreactive protein which does not contain enkephalin

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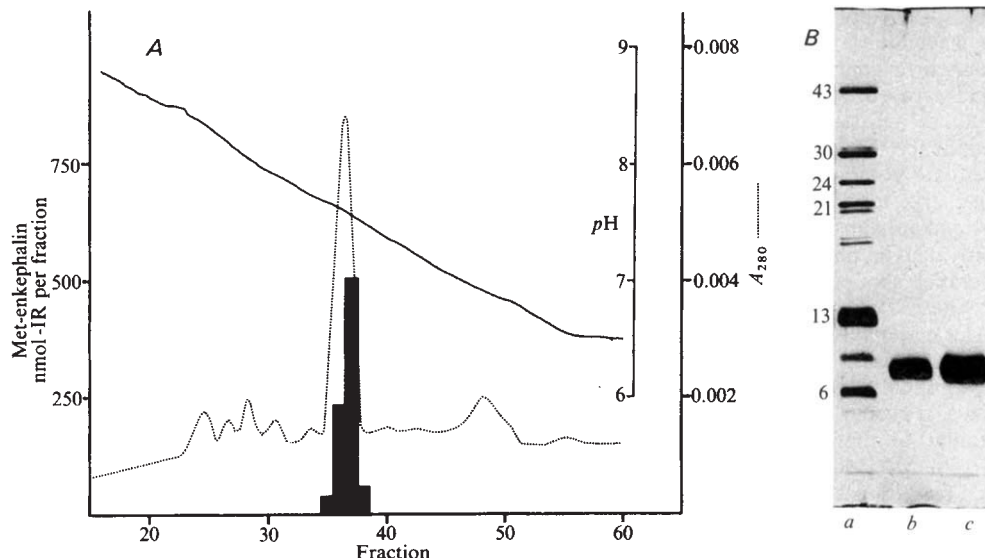
The primary sequence of adrenal proenkephalin has recently been reported by three groups^{1–3} who have isolated and sequenced the cDNA for this prohormone. Several intermediates in the processing of proenkephalin, containing from one to four copies of [Met]enkephalin, have been purified from the adrenal medulla^{4–9}. Although there is evidence that the proenkephalin is identical in the brain and the adrenal medulla, similar intermediates have not been isolated from brain. We report here the production of an antiserum directed against a purified enkephalin precursor derived from the amino terminus of adrenal proenkephalin which cross-reacts with an antigen in brain. The immunoreactive protein in brain does not contain the sequence of enkephalin, but shows a pattern of distribution in immunohistochemical studies parallel to that of the enkephalins. In extracts of bovine caudate-putamen, this antigen is present in a molar concentration approximately one-fifth of that of [Met]enkephalin. The results demonstrate that the antiserum recognizes antigenic determinants within the N-terminal 72 amino acid residues of adrenal proenkephalin and that the enkephalin precursor in brain is similar to that found in the adrenal medulla. Furthermore, the absence of the enkephalin sequence in the brain protein indicates that concentrations of the larger intermediates in the processing of proenkephalin are much lower in the brain than in the adrenal medulla.

The antigen used to produce the antiserum was prepared from bovine adrenal chromaffin granules by gel exclusion and ion-exchange chromatography (Fig. 1). The protein was followed through the purification procedure by radioimmunoassay (RIA) for [Met]enkephalin after sequential digestion of column fractions with trypsin and carboxypeptidase B^{10,11}. This digestion procedure liberates enkephalin sequences appearing within the precursor proteins but does not destroy the enkephalins themselves, and is a standard method for assaying these proteins¹². Analysis by HPLC of the purified protein after digestion with trypsin alone revealed the presence of free [Met]enkephalin. The addition of carboxypeptidase B to the digestion procedure did not increase the amount of [Met]enkephalin recovered, demonstrating that the protein contains a single copy of this peptide at its carboxy-terminus. The protein migrated as a single band on SDS-polyacrylamide gel electrophoresis with an apparent molecular weight (M_r) of 8,000 (Fig. 1B). Thus, in terms of [Met]enkephalin content, M_r on gel electrophoresis and amino acid composition (Table 1), this protein seems to be identical to an enkephalin-containing protein isolated from the adrenal medulla by Lewis *et al.*⁵, and represents the N-terminal 77 amino acid residues of bovine adrenal proenkephalin.

Antisera were produced in two Bourgoigne fawn rabbits by injecting 10 nmol of the antigen mixed with Freund's complete adjuvant bilaterally into the popliteal lymph nodes. Animals were boosted 3 and 5 weeks later in the same conditions. Sera from both rabbits obtained 2 weeks after the third injection were tested for their ability to bind the adrenal antigen labelled with ¹²⁵I. One of these sera (serum 6-II) was further character-

Fig. 1 A, Purification of the [Met]enkephalin-containing protein from the adrenal medulla used for the production of antisera. **B**, Gel electrophoresis of the purified adrenal protein.

Methods: The protein was followed through the purification procedure by RIA for [Met]enkephalin following sequential digestion with trypsin and carboxypeptidase B^{7,8}. The lysate from bovine adrenal chromaffin granules was acidified to pH 4.2 and centrifuged for 90 min at 30,000g. Ammonium sulphate was added to the supernatant to a final concentration of 33% saturation and the resulting precipitate was collected by centrifugation, resuspended in 0.05 M Tris-HCl pH 7.5, containing 4 M urea, and applied to a gel exclusion column. Fractions containing a peak of [Met]enkephalin activity (apparent M_r 13,000) were pooled and applied to a column (1.0×38 cm) of PBE 94 (Pharmacia) and eluted with Polybuffer 96 (Pharmacia) in conditions which generate a linear pH gradient over the range pH 8.5–6.5. As shown in **A**, a [Met]enkephalin-containing protein eluted as a symmetrical peak, with an isoelectric pH of 7.6. Fractions 36 and 37 were pooled and passed through a Sep-Pak microcolumn (Waters Associates) to remove salts and ampholytes. The protein solution obtained was dried and resuspended in 0.025 M Tris-HCl pH 7.5, and used for immunization of rabbits. In **B**, the gel (12% polyacrylamide) was stained using the method of Morrissey²³. Lane **a**, standard proteins (250–750 ng), with the M_r s ($\times 10^{-3}$) shown at the left. Lanes **b**, **c**, 250 and 1,000 ng of the purified adrenal protein. The unlabelled bands present in lane **a** are impurities in the standard proteins revealed by the high sensitivity of the silver-stain technique.



ized and used to establish a RIA (Fig. 2). When several synthetic peptides containing the sequence of [Met]- or [Leu]enkephalin, including fragments derived from the C-terminal region of adrenal proenkephalin, were tested in the RIA, in all cases cross-reactivity was found to be $<0.01\%$. Three larger proteins derived from the N-terminal region of adrenal proenkephalin have been purified in our laboratory and tested for cross-reactivity in the RIA. Two of these contain multiple copies of [Met]enkephalin and have M_r s greater than that of the original antigen. These proteins seem to be identical to the 12,600 and 18,200 M_r enkephalin-containing proteins reported by others^{5,6}, and are intermediates in the processing of adrenal proenkephalin into active peptides. The third protein does not contain [Met]enkephalin, but the amino acid composition is similar to that found in the N-terminal 72 amino acid residues of adrenal proenkephalin. We therefore conclude that all three proteins contain the same N-terminal sequence as the original antigen. Serial dilution of these three proteins produced binding curves in the RIA parallel to the standard curve. The extent of cross-reactivity is difficult to ascertain due to the small quantities of the proteins presently available, but it seems to be $\sim 100\%$ for all three proteins.

When the fractions from gel exclusion chromatography of an adrenal extract were analysed with this RIA, several peaks of proenkephalin-immunoreactivity (proenkephalin-IR) were found (Fig. 3a). These peaks corresponded to peaks of [Met]enkephalin activity observed after digestion with trypsin and carboxypeptidase B. However, enkephalin-containing proteins of $M_r < 10,000$ were not detected by the anti-proenkephalin serum, again demonstrating that this serum only recognizes enkephalin precursors with a M_r equal to or greater than that of the original antigen.

Because of this unique specificity, we thought that the serum would be a useful tool to study enkephalin biosynthesis in the brain. Preliminary experiments revealed substantial amounts of proenkephalin-IR in acetic acid extracts of bovine caudate nucleus. The nature of the immunoreactivity in these extracts was characterized by exclusion chromatography (Fig. 3b). In contrast to the multiple peaks of activity seen in preparations from the adrenal medulla, proenkephalin-IR eluted as a single peak. Assay of serial dilutions of this peak resulted in a binding curve which was parallel to the standard curve (Fig. 2), indicating antigenic identity of the brain protein with the adrenal

Table 1 Amino acid composition of the adrenal protein used as antigen

Amino acid	Residues per molecule	Amino acid	Residues per molecule
Asx	3.62 (4)	Leu	15.05 (14)
Thr	6.04 (7)	Tyr	2.13 (2)
Ser	5.41 (6)	Phe	1.35 (1)
Glx	10.61 (11)	His	1.33 (1)
Gly	3.21 (3)	Trp	0.86 (1)
Ala	6.03 (6)	Lys	8.62 (7)
Val	0 (0)	Arg	2.17 (2)
Met	0.90 (1)	Cys	3.79 (6)
Ile	0 (0)	Pro	4.63 (5)

Amino acid analysis of the protein (100 pmol) was performed as described by Böhlen and Mellet²². Values are the average of two determinations except for Cys and Pro, which were determined once. Numbers in parentheses refer to the amino acid composition of a protein consisting of the first 77 residues of bovine adrenal proenkephalin.

protein used as a standard. A total of 0.25 pmol of proenkephalin-IR per mg tissue was recovered in this peak.

The fractions from this column were then assayed for the enkephalins following digestion with trypsin and carboxypeptidase B. Again in contrast to the adrenal medulla, enkephalin was not found in the fractions containing the peak of proenkephalin-IR. A single peak of [Met]enkephalin immunoreactivity was found in the fractions corresponding to $M_r < 5,000$, representing the free enkephalins and enkephalin congeners. A total of 1.16 pmol [Met]enkephalin-IR per mg tissue was recovered in this peak, which, compared with the 0.25 pmol proenkephalin-IR per mg tissue found, gives a molar ratio of [Met]enkephalin-IR:proenkephalin-IR of 4.7:1, in good agreement with the ratio of 6:1 known to occur in the primary sequence of adrenal proenkephalin.

In addition to RIA, another important use of an antiserum specific for proenkephalin is the immunohistochemical localization of the antigen. As the brain protein detected by the RIA did not contain enkephalin, we were interested in comparing the regional distribution of this antigen with the well characterized distribution of the enkephalins^{13,14}. Using the immunohistochemical technique of Sternberger *et al.*¹⁵, the

anti-proenkephalin serum produced positive staining in both neuronal cell bodies and nerve terminals in several brain regions known to contain enkephalins. The hypothalamic supraoptic and paraventricular nuclei contain numerous positive magnocellular neuronal cell bodies (Fig. 4a). The external median eminence (Fig. 4b) and various central grey nuclei such as the caudate-putamen, globus pallidus, septum lateralis and bed of the stria terminalis contain positive nerve terminals. Thus, proenkephalin-IR is present not only in neuronal cell bodies but also in fibres and terminals, with a distribution which parallels that of the enkephalins.

There is growing evidence that the precursor of [Met]enkephalin in brain is identical to adrenal proenkephalin. Using antisera directed against fragments derived from the C-terminal region of adrenal proenkephalin, two groups^{16,17} have reported a distribution of immunoreactivity in brain similar to that of the enkephalins. Others¹⁸ have found a 31,000 *M_r* [Met]enkephalin-containing protein produced by cell-free translation of bovine striatal mRNA, and cDNA coding for adrenal proenkephalin has been shown to hybridize with mRNA in human caudate nucleus (M. Comb and E. Herbert,

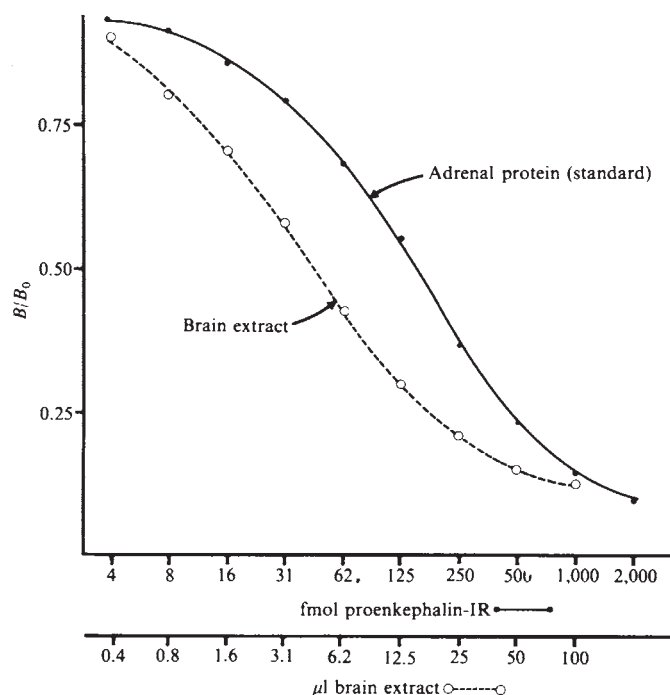


Fig. 2 Comparison of the standard curve from the proenkephalin RIA with serial dilutions of a brain extract. The incubation mixture consisted of: 100 μ l of antiserum 6-II diluted 1:4,000 in phosphate-buffered saline including 0.01% bovine serum albumin (PBSA); 100 μ l of the purified adrenal protein, labelled with 125 I by the chloramine-T method²⁴ and containing $\sim 10,000$ c.p.m., dissolved in PBSA plus 0.2% gelatin and 0.02% Triton X-100; 100 μ l of the unknown sample or standards containing 0.004–2 pmol of the purified adrenal protein as determined by amino acid analysis. The antigen–antibody complex was precipitated with IgG-sorb (Enzyme Center, Boston). Overnight incubation at 4 °C typically resulted in 20% binding of the label, which was displaced 50% by ~ 0.12 pmol of the unlabelled antigen. The label was not significantly displaced by 10 μ g of the following peptides: [Met]enkephalin, [Met]enkephalinyll-Lys, [Met]enkephalinyll-Arg, [Met]enkephalinyll-Arg-Arg, [Met]enkephalinyll-Arg-Phe, [Met]enkephalinyll-Arg-Gly-Leu, [Met]enkephalinyll-Arg-Gly-Leu-Lys, Arg-[Met]enkephalin, [Leu]enkephalin, [Leu]enkephalinyll-Lys, [Leu]enkephalin sulphate, α -endorphin, β -endorphin, γ -endorphin, α -neoendorphin, β -neoendorphin, dynorphin₁₋₈, BAM22P (ref. 7), peptide E (ref. 8), peptide F (ref. 9). Serial dilution of the brain extract purified by gel exclusion chromatography (Fig. 3b) produced a binding curve parallel to the standard curve.

personal communication). Our observation of a protein in brain which displays N-terminal adrenal proenkephalin-IR and a distribution parallel to that of the enkephalins further supports the existence of identical proenkephalins in these two tissues. However, the absence of enkephalin in the immunoreactive protein that we have isolated from brain is surprising. One interpretation of this observation is that the larger intermediates in the processing of proenkephalin do not accumulate in brain as they do in the adrenal medulla. This may indicate that the rate limiting step in enkephalin biosynthesis is not the same in the two tissues.

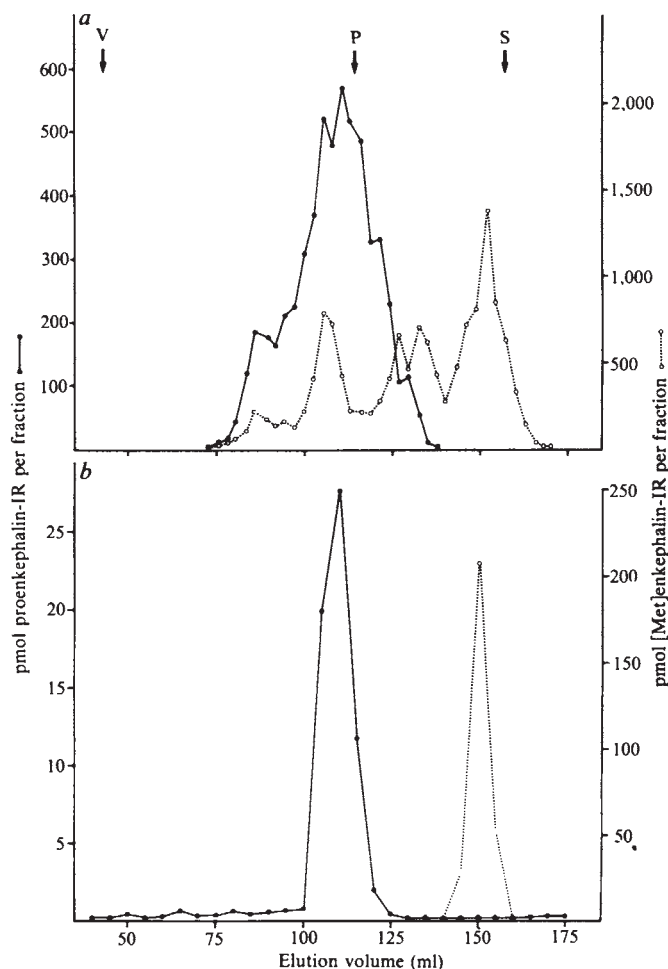


Fig. 3 Gel exclusion chromatography of acetic acid extracts of bovine adrenal medulla (a) and caudate-putamen (b). Tissues were homogenized in 4 or 5 volumes of 1 M acetic acid containing 10 μ g ml⁻¹ each of pepstatin A and phenylmethylsulphonyl fluoride and centrifuged for 2 h at 50,000g. Portions of the resulting supernatants were loaded on a column (1.6 $\times$ 90 cm) of Sephadex G-100 (Pharmacia) equilibrated with 1 M acetic acid. Aliquots of the collected fractions were lyophilized and digested with trypsin and carboxypeptidase B as described elsewhere¹¹ and the [Met]enkephalin content was determined by RIA. Separate aliquots were lyophilized, resuspended in PBSA plus 0.2% gelatin and 0.02% Triton X-100, and assayed directly with the proenkephalin RIA described in Fig. 2 legend. In contrast to the multiple peaks of proenkephalin-IR which also contain [Met]enkephalin seen in the extract of the adrenal medulla, the brain extract yielded a single peak of proenkephalin-IR that did not contain [Met]enkephalin. We tested several procedures for extracting the protein from the caudate-putamen. Extraction in 4 M urea provided a yield close to that obtained with extraction in 1 M acetic acid. Subsequent extraction of the insoluble material from these two procedures with Triton X-100 did not solubilize additional proenkephalin-IR. ●, Proenkephalin-IR; ○, [Met]enkephalin-IR. Column markers are: V, void volume; S, salt volume; P, elution volume of the purified adrenal protein from Fig. 1.

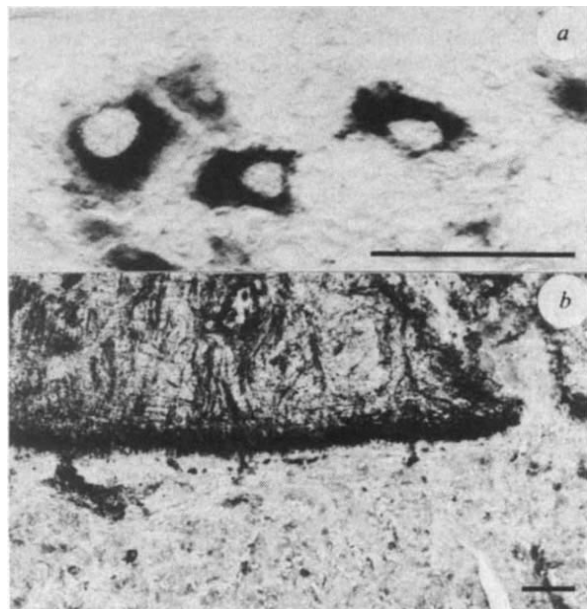


Fig. 4 Proenkephalin-IR in three neuronal cell bodies of the bovine supraoptic nucleus (a) and in numerous nerve terminals of the dog external median eminence (b). Tissue was embedded in paraffin and sections 7 μ m thick were fixed with Bouin Hollande Sublimé fixative²⁵ and stained by the peroxidase-antiperoxidase method of Sternberger *et al.*¹⁵, using a 1:2,000 dilution of serum 6-II. The positive immunoreactive material shows black. It remains positive after adsorption of the antiserum with vasopressin, oxytocin, [Met]enkephalin, [Leu]enkephalin or cholecystokinin 1-8S but becomes negative after adsorption with adrenal proenkephalin. Similar patterns of staining were observed in the brains of rat (B. Bloch, personal communication). Scale bar, 50 μ m.

The visualization of N-terminal proenkephalin-IR in neuronal fibres and terminals demonstrates that the N-terminus is transported out of the cell body. Immunohistochemical methods do not allow us to conclude whether this immunoreactivity is in the form of large intermediates in the processing of proenkephalin or in the N-terminal fragment itself. Nevertheless, our ability to isolate a protein which does not contain the enkephalin sequence but possesses N-terminal proenkephalin-IR suggests that the N-terminal fragment of brain proenkephalin is a metabolically stable molecule, and that the N-terminal fragment accompanies the enkephalins to the nerve terminal. We propose to call this molecule synenkephalin, the prefix syn- indicating that the protein appears with the enkephalins.

Synenkephalin is not the only case where a large fragment of a prohormone survives the processing of the precursor into the active hormone. Other examples include the N-terminal fragment of proopiomelanocortin¹⁹, peptide C derived from proinsulin²⁰, and the neurophysins associated with vasopressin and oxytocin²¹. This phenomenon may thus represent a general feature of peptide hormone biosynthesis.

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Histamine plays a part in induction of drinking by food intake

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Drinking occurs around meal time in most mammals. Food-related drinking accounts for ~70% of daily fluid intake for rats^{1,2}, but little is known of the mechanisms by which eating elicits drinking³. That eating⁴ and vagal stimulation^{5,6} elicit the release of histamine from gastric mucosa, together with the fact that drinking elicited by eating⁷ or exogenous histamine⁸⁻¹⁰ depends on an intact abdominal vagus, suggests a role for endogenous histamine as a component of food-related drinking in the rat. I report here that the combined antagonism of peripheral H₁ and H₂ receptors for histamine (1) attenuates drinking elicited by normal food-contingent stimulation of the gastrointestinal tract and (2) abolishes drinking elicited by pregastric food-contingent stimulation during sham feeding in the rat.

Because drinking elicited by systemic exogenous histamine is inhibited by antagonism of peripheral H₁ and H₂ receptors^{11,12}, extensive dose-response studies were performed to determine the lowest doses of antagonists which would specifically abolish drinking elicited by histamine. Dexbrompheniramine (DXB) and cimetidine were chosen as respective H₁ and H₂ antagonists because each has been shown to have a considerable degree of specificity for inhibiting drinking elicited by histamine^{9,12}. The combination of 1 mg per kg DXB and 16 mg per kg cimetidine intraperitoneal (i.p.) 10 min before histamine abolishes drinking in response to subcutaneous (s.c.) doses of histamine diphosphate (1.25-20 mg per kg) which normally elicit, in a dose-related manner, up to 13 ml of drinking in a 1-h test¹³. Larger combined doses of DXB and Hcimetidine also abolish drinking after exogenous histamine, but they seem not to do so specifically because such larger doses also inhibit ingestion in response to non-histaminergic stimuli. Thus, the finding that 1 mg per kg DXB plus 16 mg per kg cimetidine abolishes even the copious drinking elicited by a large dose of histamine without inhibiting drinking after 24-h water deprivation (a challenge known to activate both cellular and extracellular non-histaminergic mechanisms for drinking^{3,14,15}) provides an effective specific tool for blocking the dipsogenic effect of histamine. This probe was used to assess conservatively a role for histamine in drinking around meal time.

Twelve male Sprague-Dawley albino rats (300–400 g) were maintained on a 12:12 light/dark cycle with food (Charles River pellets) and water continuously available. Table 1 shows that DXB (1 mg per kg) and cimetidine (16 mg per kg) abolished drinking in a 1-h test after 2.5 mg per kg histamine—the 50% effective dose (ED_{50}) for drinking in the rat⁹. (The ED_{50} was chosen to evaluate effective blockade of histamine-elicited drinking by DXB plus cimetidine in these rats, because drinking in response to the ED_{50} is not confounded by an angiotensin II component as is drinking in response to the ED_{100} ; refs 10, 13.) These doses of antagonists failed to inhibit drinking after 24-h water deprivation in the same rats (Table 1). Thus, DXB and cimetidine specifically inhibited histamine-induced drinking in these rats, as has been demonstrated elsewhere¹³.

The same rats ate food at the midpoint of the light phase after 24-h food deprivation. They ate 10 min after intraperitoneal (i.p.) 0.9% NaCl or DXB plus cimetidine. They ate a mean of 6.6 ± 0.5 ($\pm$ s.e.) g in 1 h after the control injections, and 6.2 ± 0.4 g in 1 h after DXB plus cimetidine. While histamine antagonists did not inhibit eating ($P > 0.10$), food-related drinking was significantly inhibited by 24.0% (Table 1). Analysis of co-variance showed that the significant effect of DXB plus cimetidine on drinking was independent of any nonsignificant effect on feeding ($P < 0.001$). Moreover, examination of drinking and eating in terms of ratio of water to food intake revealed that DXB plus cimetidine significantly decreased ($P < 0.05$) the ratio from a mean of 1.2 to 0.9 ml g⁻¹.

Table 1 Effect of DXB plus cimetidine on drinking

	0.9% NaCl	DXB + cimetidine
Histamine (2.5 mg per kg)	3.1 ± 0.4	$0.8 \pm 0.2^*$
Water deprivation (24 h)	10.9 ± 0.6	10.3 ± 0.5
Feeding	7.5 ± 0.5	$5.7 \pm 1.0^+$
Sham feeding	11.1 ± 2.2	$0.3 \pm 0.3^+$

Mean ($\pm$ s.e.) water intake (ml) in 1 h after i.p. injections of 0.9% NaCl or 1 mg per kg DXB plus 16 mg per kg cimetidine (see text for additional details). Drinking elicited by 2.5 mg per kg histamine after DXB + cimetidine was not different ($P > 0.20$) from drinking without injection of histamine. A matched-pair 2-tailed *t*-test was used for all within-group comparisons.

*+ Significantly different ($P < 0.001$ and < 0.01 , respectively) from drinking after 0.9% NaCl injection.

The 24% inhibition of food-related drinking is a conservative estimate of the magnitude of endogenous histamine's role. Greater inhibition of food-related drinking can be achieved with larger doses of DXB plus cimetidine, but because larger doses nonspecifically suppress ingestion of water and food, it is difficult to interpret those results. Thus, I conclude that a histaminergic mechanism accounts for at least 24% of drinking around meal time in these conditions.

Because vagal excitation is a sufficient stimulus for the release of endogenous histamine^{5,6}, sham feeding should be expected to elicit drinking in the rat, and indeed it does¹⁶. Seven male rats (475–600 g) equipped with stainless-steel gastric cannulas^{17,18} sham-fed sweet milk food after 24-h food deprivation. They sham-fed a mean of 59.8 ± 10.1 ml in 1 h when eating with fistulas open¹⁸ 10 min after 0.9% NaCl i.p., and sham-fed 52.6 ± 8.2 ml when eating 10 min after 1 mg per kg DXB plus 16 mg per kg cimetidine. While the histamine antagonists did not inhibit sham feeding ($P > 0.20$), drinking contingent on sham feeding was abolished (Table 1). Co-variance analysis showed this effect to be independent of any effect of DXB plus cimetidine on sham feeding ($P < 0.025$).

Combined antagonism of H_1 and H_2 receptors by systemic DXB plus cimetidine failed to inhibit drinking after water deprivation but abolished drinking elicited by exogenous histamine and inhibited drinking by 24% when rats ate a normal meal after food deprivation. If the combined H_1 and H_2 antagonism was successful, as these and other data^{12,13} suggest,

in completely blocking a histaminergic component of drinking elicited by peripheral histamine, the results show that a histaminergic mechanism is only partly the cause of drinking around mealtime in the rat. This conclusion is consistent with the reports that cellular dehydration^{19,20} and angiotensin II²¹ are also candidates for food-related drinking and that abdominal vagotomy, which abolishes drinking elicited by a meal⁷, inhibits drinking elicited by systemic histamine^{8–10}, cellular dehydration^{22–25} and angiotensin II^{26,27}.

That combined antagonism of H_1 and H_2 receptors abolished drinking elicited by sham feeding, suggests that the drinking elicited by pregastric food-contingent stimulation is mediated exclusively by H_1 and H_2 receptors despite the multiple neuroendocrine consequences of sham feeding in the rat^{28,29}.

Although it is tempting to conclude that such a histaminergic mechanism involves exclusively peripheral receptors for histamine of gastric origin, such a conclusion at this point would be premature. For example, while cimetidine fails to cross the blood-brain barrier³⁰, DXB should³¹. Thus, the involvement of central H_1 receptors for inhibition of drinking in these experiments cannot be ruled out. This possibility is made less likely, however, by the reports that systemic ¹⁴C-histamine is not found in the brain³² and that drinking elicited by systemic histamine¹⁰ or eating⁷ is abolished by manipulations which should not block central receptors for histamine.

The finding that histamine has a role in induction of drinking by food intake and the future identification of the mechanism involved is a step towards remedying the neglect which followed the original description¹ of the importance of eating as a stimulus for drinking in the rat. Moreover, understanding this fundamental problem may be useful given the report that drinking around mealtime and after cellular dehydration, systemic histamine and angiotensin II are disordered in the adult spontaneously hypertensive rat³³.

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Suppressor T cells control the HLA-linked low responsiveness to streptococcal antigen in man

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We have previously reported that low immune responsiveness to the streptococcal cell wall (SCW) antigen is controlled by an HLA-linked dominant gene which we designated as an immune suppression gene to the SCW antigen (*Is-SCW*) without knowing its function¹. We have extended the study of the genetic control of the immune response to the SCW antigen and confirmed both the bimodal distribution of immune responsiveness and HLA-linked dominant inheritance of low responsiveness². Here we report an analysis of the function and expression of *Is-SCW* at the cellular level and demonstrate that the *Is-SCW* controls the generation of antigen-specific suppressor T cell in low responders.

The proliferative response of peripheral blood lymphocytes (PBL) to the SCW antigen was monocyte dependent and the responding T cells were included within the Leu-2a negative and Leu-3a positive subset of T cells (Table 1). To test the HLA restriction between T cells and monocytes in the immune response to the SCW antigen, we established a method of co-culture with the minimal number of allogeneic monocytes which could present antigen but did not cause detectable mixed lymphocyte reaction (MLR). T cells could cooperate with HLA-DR identical or haploidentical allogeneic monocytes even if they did not share HLA-D specificities. But there was no detectable response if T cells were challenged with the SCW antigen in the presence of HLA-DR nonidentical monocytes even if they shared MTI antigen, which is another human Ia-like antigen distinct from HLA-DR antigen expressed on HLA-DR1, DR2, DRw6, DRw8 or DRw10 positive B cells³. Our recent study also revealed that the SB antigen, which is a newly discovered human Ia-like antigen and is encoded for by a locus mapped between *HLA-D/DR* and *GLO* (ref. 4), is not a restriction molecule for the interaction between T cells and monocytes (Fig. 1). These observations have shown that there is a genetic restriction between T cells and monocytes in the response to the SCW antigen which is controlled by the products of *HLA-DR* but not by *HLA-D*, *MTI* nor by *SB*, and that the allospecific determinant of HLA-DR molecule on monocytes was recognized by T cells. This was confirmed by the observation that the immune response was completely abolished either by the treatment of the monocytes with anti-*HLA-DR* framework monoclonal antibody and rabbit complement or by the addition of the same antibody in the culture medium. (Table 1).

T cells from high responders showed strong response to the SCW antigen in the presence of HLA-DR identical or haploidentical low responder monocytes. However, T cells from low responders failed to respond to the SCW antigen even in the presence of HLA-DR identical or haploidentical high responder monocytes (Table 2). These results suggest that low responsiveness is controlled by T cells and not by monocytes. It is interesting that there are several individuals who are HLA-DR identical so that they stimulate minimal mixed lymphocyte reaction (MLR), but who are either high or low responders to the SCW antigen. This suggests that the *Is-SCW* is not in absolute linkage disequilibrium with any *HLA-DR* allele.

Possible explanation for the failure of T cells from low responders to proliferate to the SCW antigen include: (1) inadequate sensitization of T cells *in vivo*, (2) an unknown defect on T cells such as a lack of receptors for the antigen or interleukins, and (3) the existence of suppressor T cells. To test these possibilities,

high responder T cells and monocytes were challenged with the SCW antigen in the presence of low responder T cells. As shown in Table 3, low responder T cells suppressed the responsiveness of HLA-DR identical high responder T cells. Characterization of the suppressor T cell using monoclonal antibodies to Leu-2a and Leu-3a markers⁵ revealed that the Leu-2a positive but Leu-3a negative T-cell subpopulation had strong suppressive activity whereas Leu-2a negative and Leu-3a positive T cell had no effect on the immune responsiveness of the T cell from the HLA-DR identical high responder. To summarize our findings to date, the suppressor T cell had the following features: (1) nylon wool and plastic dish nonadherent, (2) HLA-DR negative, (3) surface immunoglobulin negative, (4) Leu-1 positive, (5) Leu-2a positive and (6) Leu-3a negative. By knowing these features of the suppressor T cell, it was possible to eliminate the Leu-2a positive suppressor T cell, from low responder T cells. As shown in Table 3, low responder T cells did respond strongly to the SCW antigen after elimination of Leu-2a positive and Leu-3a negative T cell by using panning methods⁶ with monoclonal antibodies to Leu-2a or Leu-3a. These observations imply that low responders had all the T-cell subsets necessary for the response to the SCW antigen and failure to respond to the SCW antigen was not because of a failure to be sensitized by this antigen *in vivo*, but because of the existence of active suppressor T cells. Thus, low responsiveness to the SCW antigen is controlled by active suppressor T cell, and these results confirm our designation of the HLA-linked dominant gene responsible as an immune suppression gene (*Is* gene).

Association between low responsiveness to tetanus toxoid⁷ and schistosomal antigen⁸ with the HLA haplotype (HLA-Bw52-Dw12-DR2) might be explained by the existence of HLA-linked *Is* genes in man. It is conceivable that dominant *Is* genes contribute to the normal state of tolerance to self antigens or many allergens. If so, the absence of such genes in a homozygous individual might predispose to an undesirable

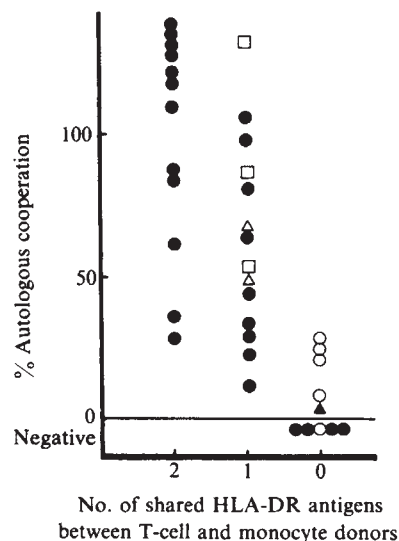


Fig. 1 Genetic restriction by HLA-DR antigen in the cooperation between T cell and allogeneic monocyte in the immune response to the SCW antigen *in vitro*. T cells (7×10^4) from high responders were co-cultured with allogeneic monocytes (7×10^3 or 3.5×10^3) in the presence of $1 \mu\text{g}$ of the SCW antigen for 7 days. Per cent autologous cooperation was calculated by the following formula:

$$\frac{\Delta \text{ c.p.m. observed in T cells and allogeneic monocytes}}{\Delta \text{ c.p.m. observed in T cells and autologous monocytes}} \times 100$$

Δ c.p.m. was calculated by subtracting median control c.p.m. (without antigen) from median experimental c.p.m. (with antigen). $\square$, HLA-DR haploidentical but HLA-D nonidentical; $\circ$, HLA-DR nonidentical but sharing MTI; $\triangle$, HLA-DR haploidentical but SB nonidentical; $\blacktriangle$, HLA-DR nonidentical but SB haploidentical; $\bullet$, informative only for the sharing of HLA-DR.

Table 1 Characterization of the proliferative response of PBL to the SCW antigen, *in vitro*

Cell donor (HLA-DR)	Cells	Antibody	SCW antigen (c.p.m.)	Medium (c.p.m.)
N.O. (DR2, DRw6)	PBL 1×10^5	—	75,026	4,684
	T cells 7×10^4	—	1,051	281
	Monocytes (M) 7×10^3	—	1,363	669
	T cells + M	—	86,521	1,488
	T cells + M*	NMIG + C	84,626	777
	T cells + M*	HU-4 + C	1,051	236
	T cells + M	NMIG†	57,281	5,221
	T cells + M	HU-4†	2,371	1,272
	Leu-2a ⁺ 3a ⁻ T cells + M	—	8,496	527
	Leu-2a ⁻ 3a ⁺ T cells + M	—	87,338	2,125
Y.N. (DR4, DR4)	PBL 1×10^5	—	39,924	2,752
	T cells 7×10^4	—	245	301
	M 7×10^3	—	218	246
	T cells + M	—	34,726	2,022
	T cells + M*	NMIG + C	48,137	831
	T cells + M*	HU-4 + C	1,537	374
	T cells + M	NMIG†	30,918	5,658
	T cells + M	HU-4†	2,481	1,170
	Leu-2a ⁺ 3a ⁻ T cells + M	—	9,531	2,921
	Leu-2a ⁻ 3a ⁺ T cells + M	—	37,147	1,579
T.S. (DRw9, DR-)	PBL 1×10^5	—	80,957	2,006
	T cells 7×10^4	—	556	430
	M 7×10^3	—	1,261	701
	T cells + M	—	73,671	1,692
	T cells + M*	NMIG + C	73,485	1,397
	T cells + M*	HU-4 + C	1,938	250
	T cells + M	NMIG†	69,565	1,692
	T cells + M	HU-4†	1,958	1,795
	Leu-2a ⁺ 3a ⁻ T cell + M	—	899	321
	Leu-2a ⁻ 3a ⁺ T cell + M	—	92,367	5,123

PBL were separated on Ficol1-Conray density gradient, and plastic dish adherent cells are defined as monocytes (M). T cells were purified by the treatment of nylon wool passed fraction of PBL with anti HLA-DR framework monoclonal antibody (HU-4) and rabbit complement. T-cell subsets were separated by the panning method⁶ using anti-Leu-2a (anti-human cytotoxic/suppressor T cell) monoclonal antibody⁵ (Becton Dickinson). T cells or monocytes were adjusted to 7×10^4 per well or 7×10^3 per well respectively for their co-culture. Cells were challenged with 1 µg of the SCW antigen in triplicate in a flat-bottomed microtitre plate with 0.2 ml of RPMI-1640 medium supplemented with 100 U ml⁻¹ penicillin, 100 µg ml⁻¹ streptomycin, 2 mM L-glutamine and 10% heat-inactivated pooled human male sera in a humidified atmosphere of 5% CO₂ and 95% air at 37 °C. Purification of the SCW antigen has been described elsewhere¹. One µCi of ³H-thymidine was added to each well on day 6, and cells were collected on glass microfibre paper after a further 16 h incubation. Incorporation of ³H-thymidine into cells was counted by a liquid scintillation counter.

* Monocytes were incubated with 50 µg ml⁻¹ of purified HU-4 or normal mouse IgG (NMIG) for 30 min at 37 °C and with rabbit complement for 30 min at 37 °C to eliminate HLA-DR positive monocytes.

† HU-4 or NMIG were added to the culture at the concentration of 5 µg ml⁻¹.

Table 2 Co-culture of T cells and monocytes from high or low responders to the SCW antigen

T-cell donor (HLA-DR)	Immune response of T-cell donor	Monocyte (M) donor (HLA-DR)	Immune response of M donor	Shared HLA-DR antigens between T-cell and M donors	SCW antigen (c.p.m.)	Medium (c.p.m.)	Δ c.p.m.
M.B. (DR1, DR5)	High	M.B. (DR1, DR5)	High	Autologous	29,519	286	29,233
		K.M. (DR1, DR5)	Low	DR1, DR5	58,675	2,885	55,790
		K.T. (DR1, DRw9)	Low	DR1	27,015	3,077	23,938
K.M. (DR1, DR5)	Low	K.M. (DR1, DR5)	Low	Autologous	867	596	271
		M.B. (DR1, DR5)	High	DR1, DR5	513	493	20
K.T. (DR1, DRw9)	Low	K.T. (DR1, DRw9)	Low	Autologous	2,601	1,310	1,291
		M.B. (DR1, DR5)	High	DR1	4,219	978	3,241
N.O. (DR2, DRw6)	High	N.O. (DR2, DRw6)	High	Autologous	20,099	628	19,471
		Y.A. (DR2, DRw6)	Low	DR2, DRw6(DEn)*	11,949	374	11,575
Y.A. (DR2, DRw6)	Low	Y.A. (DR2, DRw6)	Low	Autologous	1,853	404	1,449
		N.O. (DR2, DRw6)	High	DR2, DRw6(DEn)*	2,235	620	1,615
N.O. (DR2, DRw6)	High	N.O. (DR2, DRw6)	High	Autologous	20,161	1,350	18,811
		Y.K. (DR1, DR2)	Low	DR2	24,481	8,169	16,312
Y.K. (DR1, DR2)	Low	Y.K. (DR1, DR2)	Low	Autologous	895	460	435
		N.O. (DR2, DRw6)	High	DR2	10,855	2,952	7,903
K.W. (DR2, DRw9)	High	K.W. (DR2, DRw9)	High	Autologous	28,685	922	27,763
		G.K. (DR2, DRw9)	Low	DR2, DRw9	35,531	1,873	33,658
G.K. (DR2, DRw9)	Low	G.K. (DR2, DRw9)	Low	Autologous	1,320	32	1,288
		K.W. (DR2, DRw9)	High	DR2, DRw9	5,726	2,608	3,118

T cells (7×10^4) and autologous or allogeneic monocytes (7×10^3) from high or low responders, who shared at least one HLA-DR antigen, were co-cultured in the presence or absence of 1 µg of the SCW antigen in a flat-bottomed microtitre plate for 7 days.

* HLA-DEn is a new HLA-D specificity which is serologically associated with a split of DRw6(6y), MT1 and MT2.

Table 3 Existence of Leu-2a⁺3a⁺ suppressor T cell in low responders to the SCW antigen

Culture	Experiment 1				Experiment 2			
	SCW antigen (c.p.m.)	Medium (c.p.m.)	Δ c.p.m.	% Suppression	SCW antigen (c.p.m.)	Medium (c.p.m.)	Δ c.p.m.	% Suppression
High T cell + auto. monocyte(M)	87,305	2,039	85,266		86,521	1,488	85,033	
+ Low T cell	63,244	32,159	31,085	63.5	46,869	20,417	26,452	68.9
+ Low Leu-2a ⁺ 3a ⁺ T cell	5,875	733	5,142	94.0	21,954	7,789	14,165	83.3
+ Low Leu-2a ⁺ 3a ⁺ T cell	100,134	16,921	83,213	2.4	136,928	26,705	110,223	-29.6
Low T cell + auto. M	642	358	284		1,480	744	736	
Low Leu-2a ⁺ 3a ⁺ T cell + auto. M	572	435	137		721	546	175	
Low Leu-2a ⁺ 3a ⁺ T cell + auto. M	30,829	1,648	29,181		19,661	1,168	18,493	

Unfractionated T cells (7×10^4) from a high responder were cultured with autologous monocytes (7×10^3) and unfractionated T cells or T-cell subsets (7×10^4) from a low responder in the presence of $1 \mu\text{g}$ of the SCW antigen for 7 days. The response of unfractionated T cells (7×10^4) or T-cell subsets (7×10^4) with autologous monocytes (7×10^3) from the low responder was also tabulated. T-cell subsets from the low responder were separated by the panning method using anti-Leu-2a monoclonal antibody. Leu-2a⁺3a⁺ T-cell subset of the low responder obtained by the panning method using anti-Leu-3a monoclonal antibody also showed strong suppressive activity (data not shown) indicating that the immune suppression was not due to the effects of monoclonal antibody bound to the surface of T cells. The HLA type of the cell donors were: high responder, HLA-Aw24, Aw33, Bw52, Bw44, Cw-, DR2, DRw6, MT1, MT2, Dw12, DEn, SB4, SB5; low responder, HLA-A11, Aw33, Bw39.2, Bw44, Cw-, DR2, DRw6, MT1, MT2, Dw2, DEn, SB5, SB-.

autoimmune or allergic status in man as suggested in Graves' disease⁹, post-schistosomal liver cirrhosis¹⁰ and allergy to cedar pollen (M. Muto, Y. Saito & T.S., manuscript in preparation).

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The arrangement of myosin heads in relaxed crab muscle

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To understand the mechanism of muscular contraction^{1,2} and its regulation by Ca^{2+} (ref. 3), it is important to know the arrangement and configuration of the myosin heads in the space between thin and thick filaments in relaxed muscle. This information is still lacking, mainly because the myosin reflections in the X-ray diffraction patterns are sampled by the myofilament lattice, which makes it difficult to deduce information about the configuration of the head. In crab muscle, however, unsampled myosin reflections can be obtained, and lattice spacing (between adjacent thick filaments) can be changed by osmotic pressure applied to chemically skinned single fibres. The lattice spacing affects the intensity profiles of the myosin reflections, indicating a change of configuration of the heads. The results presented here are consistent with a model in which the myosin heads are arranged in a helical manner with a 4-fold rotational symmetry around the thick filament axis. Each head is elongated, probably 16-18 nm long and 3-4 nm thick, and is, in living resting muscle, centred 13.5 nm from the mean position of the axis of the nearest thin filament, implying that the tip of the head is very close to the surface of the thin filaments.

In X-ray diffraction patterns from crab leg muscle in the relaxed state (Fig. 1A), the features which are assigned to myosin heads are meridional reflections observed at orders of 14.5 nm and off-meridional reflections at axial spacings of 33.2 and 25.7 nm. None of the reflections are sampled by the myofilament lattice, in contrast to vertebrate skeletal muscle and insect flight muscle. The intensity distribution along the

14.5-nm reflection is that expected from a zero-order Bessel function (J_0); it has an intensity maximum on the meridian and two clear subsidiary peaks at the positions expected from a J_0 . In addition, the 7.3 nm reflection (the 2nd order of 14.5 nm) also has one subsidiary peak at about the same radial coordinate as that of the 14.5 nm reflection. The off-meridional reflections at 33.2 nm and at 25.7 nm have clear off-meridional peaks at radial coordinates similar to each other. These reflections are considered to represent the cylindrically averaged diffracted intensities arising mainly, if not exclusively, from the head arrangement around each thick filament.

The rotational symmetry of the head arrangement can be deduced from the ratio of the radial coordinate for the first subsidiary peak and that for the off-meridional peak at 25.7 nm, since the former is approximately proportional to the inverse of the distance (r_0) of the centre of gravity of the heads from the thick filament axis, while the latter is dependent on r_0 as well as N , where N refers to the N -fold rotational symmetry of the arrangement. The ratios which were measured over a wide range of a are in good agreement with the value expected from $N=4$ (Fig. 2). Computer modelling experiments show that, within this range of a , a change of configuration of the heads would have little effect on the radial coordinates. The knowledge of the rotational symmetry, combined with the measured axial coordinates of the reflections, enables us to construct the helical net of the arrangement (Fig. 3), the absolute hand of which is still to be determined. The net shows that 4 structural units are included every 14.5 nm. It is reasonable to suppose that each unit consists of two heads since weighing the crab thick filaments by scanning transmission electron microscopy shows that the mass per 14.5 nm, after correction for the paramyosin content, is equivalent to 4 myosin molecules (W. Hofmann and R. Freeman, personal communication). The assumption is also consistent with the model for the packing of myosin molecules in the thick filament proposed by Wray⁴. The thick filaments of crab muscle, with an outer diameter of 19.5-20 nm^{5,6}, could accommodate 12 ($=3N$) protofilaments spaced by 4 nm.

From the relationship between a and r_0 (Fig. 4), some information about the position, shape and size of the heads is obtained. When a single fibre is chemically skinned, the fibre swells from $a = 60$ to 72 nm. The lattice can be compressed by adding high molecular weight random polymers⁷ to the solution outside the fibre. Compressing the fibres to $a = 63$ nm is accompanied by little decrease in r_0 while, from 63 to 48 nm, r_0 is linearly reduced as a is decreased. When the lattice spacing is smaller than $a = 48$ nm, further compression does not cause further reduction in r_0 . Intensity profiles of the myosin reflexions, especially the presence of clear and deep intervening minima on the 14.5 nm layer-line, obtained from fibres at $a > 63$ nm, indicate that elongated heads originate on the thick

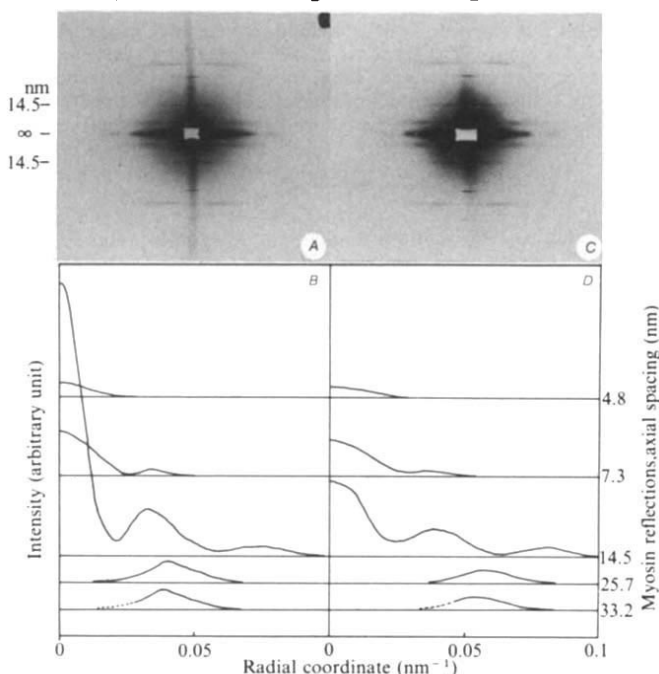


Fig. 1 A, C, X-ray diffraction patterns from single fibres from crab leg muscle; B, D, intensity profiles of the myosin reflections in A and C, respectively. A and B are from a fibre in the normal relaxing solution without dextran T-500; C and D are from a fibre in the normal relaxing solution with 4.5% dextran T-500⁷. In B and D, ordinates are scaled relative to the peak of the 5.9 nm actin layer-line. Comparing C and D with A and B, the radial positions of the two subsidiary peaks on the 14.5 nm layer-line, a subsidiary peak on the 7.3 nm layer-line and off-meridional peaks on the 25.7 and 33.2 nm layer-lines are farther away from the meridian. This shows that the heads are closer to the thick filament axis. Intervening minima on the 14.5 nm and on the 7.3 nm layer-lines are less distinct, resulting in featureless profiles, which is explained by further tilting of the heads. Off-meridional peaks on the 33.2 and 25.7 nm layer-lines are weaker, showing an additional slewing of heads. The meridional intensity of the 14.5 nm reflection becomes weaker by a larger amount than the 7.3 nm meridional one does, which cannot be explained solely by tilting, nor by slewing, but may be explained either by a separation of the two heads of myosin in the axial direction, by a negative contribution of the shaft of thick filament, or by a lattice effect of aligned thick filaments. The first explanation seems most likely, since the ratio of meridional intensity at 14.5 nm and that at 7.3 nm reverses and then returns again as the lattice becomes smaller (data not shown), which is consistent with, but does not prove, gradual separation of the two heads.

Methods: Leg muscle was dissected from crab *Portunus puber*, and single fibres were isolated after treatment in the skinning solution at 4°C for 3 h. The relaxing solution contains (in mM): KCl, 80; EGTA, 4; ATP, 4; NaN_3 , 2; PIPES, 10 at pH 7.2. The skinning solution contains 0.2% Triton X-100 in addition. An Elliott GX-18 rotating anode X-ray generator was used with conventional mirror-monochromator cameras. Exposure times were about 16 h. During this time fibres remained at constant lattice spacings which were measured by additional short exposures using a double mirror camera²². The lattice spacing measured were 64.5 nm for A and C, and 54.0 nm for B and D. Sarcomere lengths were 5.5 – 6.0 μm .

filament with a small tilt of some 20° away from perpendicular to the filament axis (see below). As the lattice is compressed (Fig. 1C,D), the meridional peak at 14.5 nm is reduced in intensity more than the subsidiary peaks, and intervening minima become less distinct, resulting in a featureless profile of the layer-line. The off-meridional reflexions become weaker. These features are explained by axial tilting and azimuthal slewing of the heads (ref 8, and present work).

The results could be interpreted in several different ways. However, the simplest and most plausible explanation is the following. (1) In fibres swollen beyond the normal dimensions, heads will not move out beyond the upper limit of $r_0 = 19$ nm. (2) At lattice spacing smaller than 63 nm, the heads can be pushed back by the surrounding thin filaments, without specific interaction with the thin filaments, as indicated by the absence of the rigor diffraction pattern^{9,10} (that is, the actin-based layer-lines, which are much intensified and extended towards the meridian compared with the relaxed patterns). There appears to be simple steric hindrance between the heads and the thin filaments. Supporting this idea, stretching muscle fibres to reduce overlap between thin and thick filaments leads to a larger r_0 (Fig. 4), indicating that the heads tend to return to their original positions, if they are not blocked by the thin filaments. In the stretched fibres, the majority of the heads are supposed to be pushed back by adjacent thick filaments, or heads. (3) Live relaxed fibres at normal sarcomere length give approximately $a = 60$ nm and $r_0 = 18$ nm. At this lattice spacing, r_0 has the same value whether thin filaments are present or not,

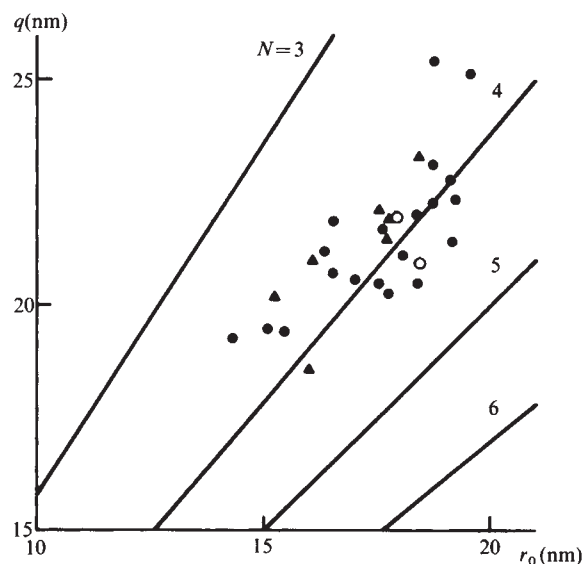


Fig. 2 Relation between the radial spacing (q) of the intensity peak on the 25.7 off-meridional reflection and the distance (r_0) of the centre of gravity of the head from the thick filament axis. r_0 is calculated from the radial coordinate (R_0) of the first subsidiary peak on the 14.5 nm layer-line as $2\pi r_0 R_0 = 3.8$. Straight lines indicate values which are expected from N -fold rotational symmetry of the arrangement of heads. Note that all the data points fall closer to the line for $N=4$ irrespective of lattice spacings, sarcomere lengths (L) and skinned (filled symbols) or live relaxed fibres (open symbols). Each point indicates the values of an individual fibre; skinned relaxed fibre at $L = 5.5$ – 6.0 μm (●) or at $L = 8.6$ – 9.2 μm (▲), live relaxed fibre at $L = 6.0$ μm in normal Ringer's solution (○). To alter r_0 , the amount of PVP K-30, dextran T-70 or dextran T-500 added was altered. Fibres with $r_0 < 14$ nm give rise to the off-meridional reflections whose peaks are too weak to be recognized. X-ray patterns were obtained as described in the legend for Fig. 1 except for patterns from live relaxed muscle, which were taken by the mirror-monochromator camera^{22,23} at beam line X-11, EMBL/Hamburg, using synchrotron radiation from the storage ring DORIS, operated at 3.3 GeV, 30 – 40 mA. The beam with a wavelength of about 0.165 nm was focused to a point on a film at a distance of 2 m behind the specimen. Freshly dissected fibre bundles were perfused with oxygenated Ringer's solution at 10°C ⁹. Exposure times were 10 – 30 min.

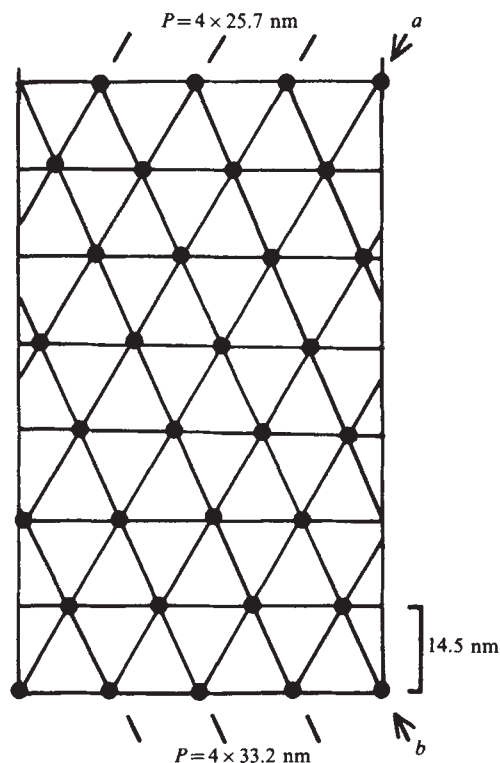


Fig. 3 The helical net of the arrangement of myosin heads around the thick filament. Levels (horizontal lines) where four structural units (●) are situated are axially spaced by 14.5 nm. All the units are connected either by a set of four identical helices (one of them is indicated by arrow (a) with the axial separation of 25.7 nm and the pitch of 102.8 nm ($=4 \times 25.7$), or by another set of four identical helices (arrow b) with the axial separation of 33.2 nm and the pitch of 132.8 nm ($=4 \times 33.2$). The structure repeats itself approximately after 101.5 nm in the axial direction. Each unit is believed to represent one myosin molecule, that is, two heads.

The absolute hand is not yet known.

while at $a < 60 \text{ nm}$, r_0 differs at the two sarcomere lengths. In other words, the interactions which affect the configuration of heads takes place at $a < 60 \text{ nm}$. Moreover, considering the mode of packing of thin and thick filaments in the filament lattice of the crab muscle⁵, it is calculated that the myosin head is centred at 13.5 nm from the mean position of thin filament axis. This implies that, in live relaxed muscle, the tip of the head is situated very close to the surface of the thin filament. (4) The lower limit of $r_0 = 11.5 \text{ nm}$ can be explained if it is supposed that the heads have already been fully pushed back and tilted down on the surface of the thick filaments and further compression results in solely further reduction in distance between the head and the thin filaments.

Considering the diameter (19.5–20 nm) of the thick filaments of crab muscle and the maximum (19 nm) and the minimum (11.5 nm) value of r_0 , it can be deduced that the elongated head is 3–4 nm thick and not longer than 18 nm, and probably in the region of 16 nm. The interaction takes place at lattice spacing smaller than $a = 60 \text{ nm}$, affecting the configuration of the head, and moreover the rigidity of the fibre against compression shows a sharp increase at $a = 60 \text{ nm}$ in plots of lattice spacing against osmotic pressure (detailed data will appear elsewhere). It is suggested that a reduction in lattice spacing from 19 to 18 nm might not cause substantial tilting of the 16 nm long heads.

The elongated myosin heads, 16–18 nm long, 3–4 nm thick and tilted about 20° , are consistent with intensity profiles of the myosin reflexions from fibres with $a > 63 \text{ nm}$. Nevertheless, the figures should not be taken without reservation. Computer simulation experiments show that the intensity profile is dependent on both the shape and the size of heads; the profile can be explained equally either by rod-shaped heads of this size or by pear-shaped heads^{11,12} with larger thickness (details of the

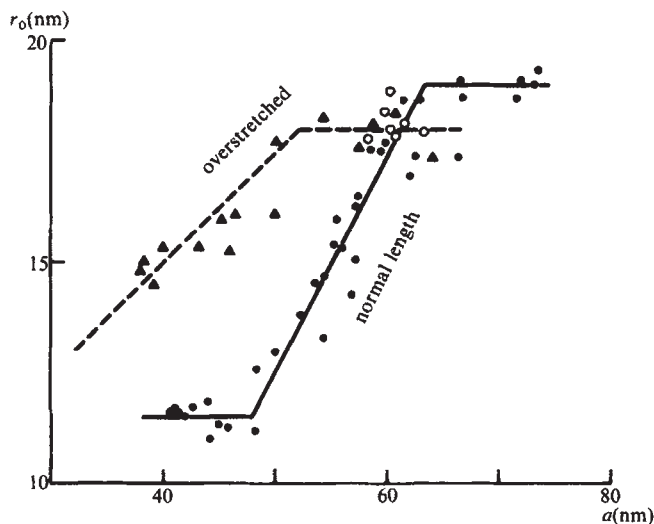


Fig. 4 Relation between the distance (r_0) of the centre of gravity of myosin head from the thick filament axis and the lattice spacing (a). r_0 and a are obtained from X-ray diffraction patterns as described in the legend for Fig. 2 and Fig. 1, respectively. Each point indicates values of an individual fibre. Symbols are the same as in Fig. 2. The solid line, consisting of three parts, fits values of fibres at normal sarcomere length, $L = 5.5$ – $6.0 \mu\text{m}$. The broken line, consisting of two parts, fits values of overstretched fibres of $L = 8.6$ – $9.2 \mu\text{m}$. r_0 is not obtained from overstretched fibres at $a < 39 \text{ nm}$ because of pronounced sampling of the 14.5 nm layer-line by aligned thick filaments. The decrease of r_0 by addition of polymers is due to physical compression of the lattice rather than due to chemical effects of the polymers, since polyvinylpyrrolidone and dextran produce the same effects. The values of r_0 as well as a are fully reversible when fibres are returned to the polymer-free relaxing solutions.

computer simulation experiments are to appear elsewhere). Moreover, Fig. 4 has been interpreted assuming direct contact between the head and adjacent filaments. Actually, the interaction might be a long range one.

Although the nature of the interaction is not known, it is of interest to make the following two points. First, the slope of the plot of a against r_0 for the fibres at normal length indicates that the centres of the heads are kept at a constant distance, 13.5 nm, from the mean position of the thin filaments, while r_0 is varied substantially. On the other hand, the slope for stretched fibres, which is half as steep as that of normal fibres, is well explained if one assumes a tip-to-tip interaction between two heads originating from thick filaments adjacent to each other. Supporting this idea, at $a < 39 \text{ nm}$, the meridional intensity at 14.5 nm becomes narrower across the meridian, indicating lateral alignment of the thick filament.

The present results clearly show that the layer-line intensity profiles, and therefore the configuration of the heads, are very dependent on lattice spacing. Accordingly, caution is needed when observing effects of various media and ATP analogues on the configuration of the heads. Thus, pH, ionic strength, Mg^{2+} concentration affect the lattice spacing strongly (data not shown). Stretching frog skeletal muscle beyond $2.6 \mu\text{m}$ results in a decrease in intensity of the 43.9 nm off-meridional, as well as of the 14.3 nm meridional reflexion^{13,14}. The weakened reflexions are recovered when the fibres are left for a day in Ringer's solution¹⁴, or when the solution is diluted¹³. The results may be explained by changes in tilting angle of the heads which are pushed back by the near-by thick filaments. Supporting this explanation, the lattice spacing is reduced when live muscle is stretched^{15,16}, and expanded by ageing¹⁷ or hypotonicity^{18,19}.

The results presented here are interpreted to mean that, in live relaxed crab muscle, the tip of the myosin heads reach the surface of thin filament. From the profile²⁰ of the 43.9 nm reflection from frog muscle (assuming 3-fold rotational symmetry of the head arrangement), the heads of frog muscle at rest length can be calculated to be centred at 11.5 to 15 nm

(depending on the azimuthal position of the heads around the thick filament) from the mean position of the thin filament axis, which is similar to that in crab muscle. It would be interesting to know whether tension generation and Ca^{2+} regulation are affected by lattice spacing and configuration of the heads. It is reported that skinned rabbit skeletal muscle exerts the highest Ca^{2+} sensitivity if the lattice spacing is the same as that of live relaxed muscle²¹.

Further quantitative analyses of the head configuration at various lattice spacings and in various media, and analyses of the mechanical properties of the heads in relaxed muscle are now in progress. For these investigations, the system described here offers two advantages, namely that the myosin reflections

are unsampled and that the configuration of the heads can be altered. In addition to this, crab muscle should also allow detection of configurational changes of the heads during contraction.

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An immunologically active chimaeric protein containing herpes simplex virus type 1 glycoprotein D

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Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) cause both persistent and latent infections, including recurrent cutaneous disease, lethal neonatal disease, central nervous system disease and other clinical syndromes¹. Modified live vaccines or conventionally prepared subunit vaccines have generally been unsuccessful in the treatment of HSV-1 and HSV-2 infections from the standpoints of safety and efficacy². It has been established that HSV-1 and HSV-2 infectivity may be neutralized *in vitro* with antisera directed specifically against each of the four major glycoproteins of the virus (gA/gB, gC, gD and gE)^{3,4} and antisera against glycoprotein gD, of either HSV-1 or HSV-2, are capable of neutralizing both HSV-1 and HSV-2 infectivity *in vitro* and *in vivo*^{5–8}. We have previously reported on the identification, DNA sequence and expression at low level in *Escherichia coli* of the gD gene of HSV-1 strain Patton⁹. Here we describe construction of a hybrid gene encoding a chimaeric protein containing HSV-1 gD, bacteriophage λ Cro and *E. coli* β -galactosidase (gD- β -gal) protein, which is expressed at high level in *E. coli*. Moreover, the chimaeric protein elicits antibodies in rabbits that not only immunoprecipitate gD from cells infected with HSV-1 and HSV-2 but also neutralize HSV-1 and HSV-2 infectivity *in vitro*.

We previously reported the construction of a plasmid, pEH25, that encodes, under control of the *lac* promoter-operator, a hybrid protein composed of 342 amino acids of the HSV-1 (Patton)gD carboxy-terminus (the entire gene encodes 396 amino acids) fused to the amino-terminal 24 amino acids of the bacteriophage λ Cro protein⁹. This hybrid protein (Cro-gD) could be immunoprecipitated by a number of monoclonal antibodies specific for gD, including one (4S) which defines a major type-common neutralizing epitope of the gD molecule⁷. However, the yield of this protein in *E. coli* was only about

0.1% of the total cell protein which was insufficient for vaccine production. We found however, that ligation of a Cro-gD amino-terminal coding region contained in pEH25 to a DNA sequence encoding *E. coli* β -galactosidase (β -gal), resulted in expression of a chimaeric Cro-gD- β -gal (gD- β -gal) protein in *E. coli* at a much higher level.

The construction of the chimaeric gD- β -gal gene is shown in Fig. 1. The gD expression plasmid pEH25 was opened at an *Nru*I site (161 base pairs (bp) downstream from the gD termination codon) and was processively digested with exonuclease *Bal*31. The *Bal*31 digestion was used to produce a population of DNA fragments with between 200 and 400 bp deleted at each terminus. This digestion thus removed the gD gene termination codon and approximately 40–240 bp of the carboxy-terminal coding region of the gD gene. The *Bal*31 treated gD coding sequence was ligated to a DNA fragment containing the β -galactosidase gene (Fig. 1b) and used to transform NF1829 [an *E. coli* strain which overproduces the *lac* repressor thus inhibiting over 95% of transcription of the gene unless specifically induced by lactose or isopropyl- β -D thiogalactopyranoside (IPTG)]. Ampicillin-resistant colonies were isolated and tested for β -galactosidase activity by replica-plating on lactose MacConkey agar indicator plates^{10,11}. Isolates producing red colonies on the indicator plates were further tested for synthesis of a chimaeric gD- β -gal protein by analysis of IPTG-induced polypeptides by SDS-polyacrylamide gel electrophoresis. From 15 such isolates, one was found to produce, on induction, significant quantities of a polypeptide of appropriate size (molecular weight 160,000, Fig. 2a). This clone contained a plasmid (pEH4-2) in which 205 bp of the carboxy-terminal gD coding sequence were deleted. Hence, the pEH4-2 gD- β -gal protein lacked the 69 carboxy-terminal amino acids of gD. The chimaeric protein was, however, immunoprecipitated by the 4S monoclonal antibody (Fig. 2b), demonstrating that the type-common neutralizing epitope of gD defined by this monoclonal was retained in this construction. The chimaeric protein specified by pEH4-2 constituted 5–10% of the total cellular protein 5 h after induction with IPTG whereas the other 14 isolates tested were found to accumulate at least 10-fold lower amounts of a chimaeric protein on induction. Plasmids contained by these other isolates were found to carry deletions removing only 30–80 bp of the carboxy-terminal gD coding sequence. These data indicate, therefore, that removal of gD coding sequences within the region 80–205 bp from the termination codon is required for efficient expression of the gene in *E. coli*. Note that this region encodes the putative transmembrane sequence of gD⁹.

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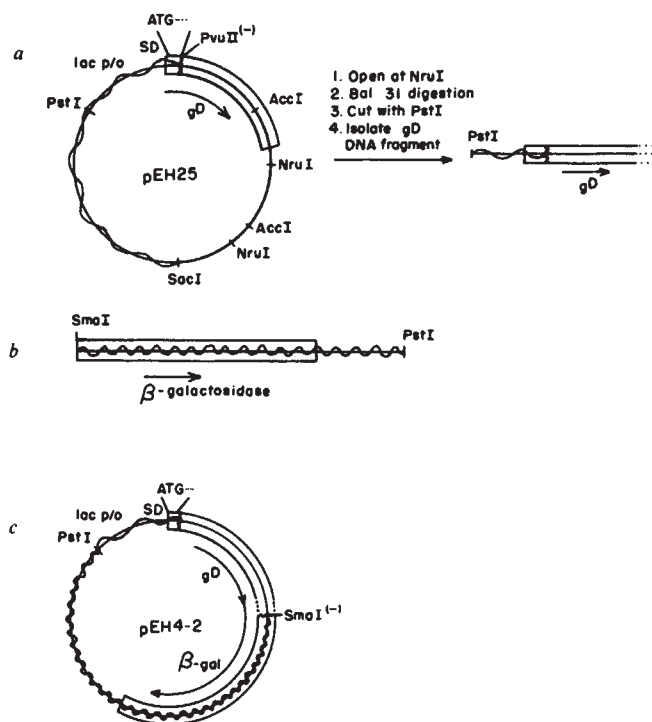


Fig. 1 Construction of plasmid pEH4-2. *a*, The preparation of DNA fragments containing variable lengths of the *cro*-gD coding sequence (represented by the open box), under the transcriptional control of the *E. coli* *lac* UV5 promoter-operator. The gD expression plasmid pEH25 (ref. 9) was digested with restriction endonuclease *Nru*I, then digested with the exonuclease *Bal*31 in conditions in which approximately 200–400 bp at each DNA terminus were removed. The plasmid was then digested with restriction endonuclease *Pst*I and DNA fragments of the appropriate size class (1,750–1,950 bp) were isolated by preparative agarose gel electrophoresis. Translation of the *cro*-gD coding sequence is controlled by the bacteriophage λ *cro* Shine-Dalgarno (SD) ribosome binding site. The direction of transcription of the gD gene is indicated by the arrow. *b*, The preparation of a DNA fragment containing the β -galactosidase coding region (represented by the open box). This was obtained from plasmid pJS413 (ref. 9 and J. Salstrom, manuscript in preparation) by digestion with *Sma*I and *Pst*I. The appropriate DNA fragment (5,532 bp) was isolated by agarose gel electrophoresis¹⁷. *c*, The ligation of two fragments from *a* and *b* above and transformation of *E. coli* strain NF1829 using the CaCl_2 procedure¹⁸. The fragments were ligated by T4 DNA ligase. Transformants were selected on LB agar plates containing 100 $\mu\text{g ml}^{-1}$ ampicillin and were replated on lactose MacConkey agar plates for a determination of β -galactosidase activity¹⁰ (that is, red coloration). Red coloration showed that the gD and β -gal coding regions were ligated in the correct reading frame, as only those colonies making a gD- β -gal chimaeric protein should contain significant β -galactosidase activity.

To test the ability of the gD- β -gal protein to elicit an antibody response to gD, the chimaeric protein was purified by SDS-polyacrylamide gel electrophoresis from extracts of induced pEH4-2 transformants, and then injected into two rabbits (018 and 019). To test for immunoprecipitation of gD, preimmune and immune sera from rabbits 018 and 019, and the 4S monoclonal antibody, were incubated with solubilized extracts of ³⁵S-methionine-labelled HSV-1, HSV-2- and mock-infected cells. The proteins precipitated by adsorption to *Staphylococcus aureus* were resolved by SDS-polyacrylamide gel electrophoresis and were visualized by fluorography. The resultant fluorograph (Fig. 3) indicates that immune sera from rabbits 018 and 019, and the 4S monoclonal antibody, precipitated gD from both HSV-1- and HSV-2-infected cells. The difference in molecular weight of type 1 and type 2 gD observed here has been described previously^{12,13}. Figure 3, which was overexposed

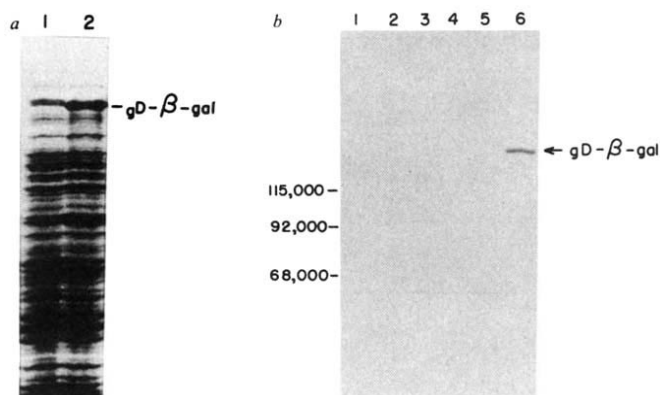


Fig. 2 *a*, Production of the pEH4-2 chimaeric protein. Duplicate pEH4-2 transformant cultures were grown to a density of 2×10^8 cells per ml. One culture was induced by adding IPTG to 1 mM (ref. 10). Both cultures were then grown for an additional 2 h at 37 °C and concentrated by centrifugation. After lysis in sample buffer (containing 3% SDS and 0.7 M β -mercaptoethanol), lysates from uninduced (1) and IPTG-induced cultures (2) were applied directly to a 7% SDS-polyacrylamide gel¹⁹. Following electrophoresis, protein bands were visualized by staining with Coomassie blue dye. *b*, Immunoprecipitation of the pEH4-2 chimaeric protein. Proteins synthesized by cultures of pEH4-2 transformants were labelled with ³⁵S-methionine either without induction (1, 3 and 5) or following induction with 1 mM IPTG (2, 4, 6) using procedures described previously⁹. Cell lysates were incubated with no added antibody (1, 2), with 1 μl of a control fibronectin-specific monoclonal antibody (3, 4) or with 1 μl of the 4S gD-specific monoclonal antibody (5, 6), and antibody-bound proteins were precipitated using *Staphylococcus aureus*²⁰ as described previously⁹. Immunoprecipitates were resolved by SDS-polyacrylamide gel electrophoresis and visualized by fluorography following treatment of the gel with sodium salicylate²¹. To the left of the fluorograph are indicated the positions of molecular weight markers (115,000, 92,000 and 68,000) co-electrophoresed with the immunoprecipitates.

Table 1 Neutralization of HSV-1 (Patton) and HSV-2(G) by antisera against gD- β -gal

	No complement		Complement	
	HSV-1	HSV-2	HSV-1	HSV-2
Rabbit 018	128	8	768	32
Rabbit 019	128	12	512	32
gD Monoclonal 4S	20,000	20,000	20,000	20,000

Sera from rabbits 018 and 019 were tested for virus neutralization in the presence of 5% complement heat inactivated at 56 °C for 30 min (No complement) or 5% active guinea pig complement (Complement)⁷. The serum dilutions which gave 50% plaque reduction, compared with the preimmune serum controls, are reported.

to show clearly the less intense HSV-2 gD band, also shows that additional proteins were specifically precipitated from HSV-1-infected cells with various rabbit sera. For example, both preimmune and immune 018 and 019 sera precipitated a protein of ~110,000 molecular weight (Fig. 3; lanes 2, 5, 8, 11). The nature of this protein is unknown, but is clearly unrelated to gD. Proteins related to authentic gD of 128,000 and 32,000–35,000 molecular weight have been reported by others to be present in extracts of HSV-1-infected cells^{14,15}.

The neutralization of virus infectivity by the gD- β -gal antisera was determined by preincubating 50–100 plaque-forming units (PFU) of HSV-1 and HSV-2 with dilutions of preimmune or immune antisera in the presence or absence of active complement⁷. After 3–4 days the HSV plaques were counted and the effect of antisera on the reduction of the number of plaques was determined. The results shown in Table 1 demonstrate that

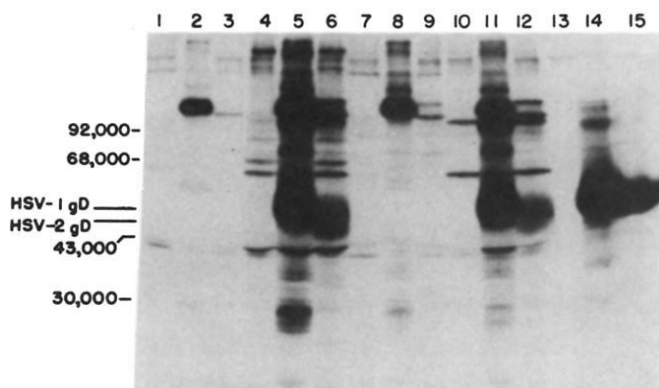


Fig. 3 Immunoprecipitation of HSV-1 and HSV-2 gD with antisera directed against the gD- β -gal chimaeric protein raised in two New Zealand white rabbits (018 and 019). Mock-infected (1, 4, 7, 10, 13), HSV-1-infected (2, 5, 8, 11, 14) and HSV-2-infected (3, 6, 9, 12, 15) cells were lysed using IP-3 buffer⁹ and cleared by centrifugation. The lysates were incubated with 10 μ l of pre-immune 018 serum (1–3), 10 μ l of immune 018 serum (4–6), 10 μ l of preimmune 019 serum (7–9), 10 μ l of immune 019 serum (10–12), or 1 μ l of the 4S monoclonal antibody (13–15). The gD- β -gal protein from pEH4-2 transformants of *E. coli* was purified by lysis of IPTG-induced cells with detergent and lysozyme followed by the extraction of the insoluble protein by repeated centrifugation (W. DeLorbe, J.H.W. and M. S. Collett, unpublished procedure). The concentrated protein was electrophoresed in 7% SDS-polyacrylamide gels and the gel slices containing the chimaeric protein were emulsified with an equal volume of Freund's complete adjuvant to immunize the rabbits. Each rabbit was injected subcutaneously with 50 μ g protein. After 21 and 31 days, antigen boosts identical to the first injection, except with Freund's incomplete adjuvant, were given. 41 days after the initial injection the animals were bled and these immune sera and preimmune sera (collected before immunization of the rabbits) were tested for immunoprecipitation of gD. Vero cells were infected with either HSV-1 (Patton) or HSV-2 (G) at a multiplicity of 10 PFU per cell, or were mock-infected, and proteins were labelled with ³⁵S-methionine for 16 h following infection. Immunoprecipitated proteins were analysed by SDS-polyacrylamide gel electrophoresis and fluorography as described in the legend to Fig. 2b. Molecular weight markers (92,000, 68,000, 43,000 and 30,000) are given on the left and were co-electrophoresed with the immunoprecipitates. The positions of the immunoprecipitated HSV-1 and HSV-2 gD polypeptides are also indicated.

antisera from each rabbit was capable of neutralizing both HSV-1 and HSV-2. Neutralization was evident in the absence of active complement, although the presence of active complement markedly increased neutralization activity. Neutralization was more effective against HSV-1 than HSV-2, which suggests that these antisera contain both type-common and HSV-1-specific neutralizing antibodies.

The results presented here demonstrate the feasibility of producing a subunit vaccine against HSV-1 and HSV-2 using recombinant DNA techniques. We have shown that this HSV gD chimaeric protein is stable and can be produced in quantity in *E. coli*. Such stability may result from the presence of flanking *E. coli* or λ sequences, or from sequestering of the fusion protein in dense, insoluble inclusion bodies¹⁶ (J.H.W., unpublished data). Further, we have demonstrated that antisera produced in rabbits in response to this chimaeric protein are capable of neutralizing plaque formation of both HSV-1 and HSV-2 *in vitro*. Experiments are now in progress to determine whether vaccination with the chimaeric protein is capable of protecting experimental animals from HSV challenge.

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Functional interrelationship between two tandem *E. coli* ribosomal RNA promoters

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The *Escherichia coli* chromosome carries seven cistrons encoding ribosomal RNA sequences^{1–3}. In all cases studied, *in vitro*^{4–7} and *in vivo*^{8–10}, it has been established that transcription is initiated from two tandem promoters. The expression of the rRNA cistrons is regulated in response to growth rate as well as to aminoacyl tRNA availability¹¹. In the present study, a plasmid (pPS1) carrying the promoter region of the *rrnA* cistron fused to the terminator region of *rrnB*¹⁰ has been used for *in vitro* transcription experiments. The presence of the terminators (T1 and T2) together with the fact that supercoiled DNA is found to be a highly efficient template, provide an ideal *in vitro* system in which to study the functional interrelationship between the two tandem promoters of *E. coli* rRNA cistrons. The results suggest that the rate of rRNA synthesis in *E. coli* cells growing in various conditions, as reflected by the availability of RNA polymerase, is primarily dependent on the properties of the two tandem rRNA promoters.

When supercoiled plasmid DNA (80% of the DNA in supercoiled form) was used as template for *in vitro* transcription using *E. coli* RNA polymerase, microgramme amounts of RNA were synthesized. Only nanogramme amounts were synthesized with the same DNA following linearization by *EcoRI* digestion at a unique site about 400 bases upstream of promoter 1 (P1)¹⁰. Experiments with mixed supercoiled and linear DNA as template revealed no effect of the linear DNA on the efficiency of supercoiled DNA as template. When small amounts of supercoiled DNA (0.1 μ g ml⁻¹) are used as template, full-length promoter (P2) transcripts appear first (Fig. 1). One minute later, at 20 °C, full-length P1 transcripts begin to appear. As can be seen in Fig. 1, the rate of appearance of full-length P1 transcripts after the first 2 min is much greater than for P2. Moreover, when P1 is transcribed at maximum speed, transcription from P2 is significantly slowed down. After 4 min of incubation the

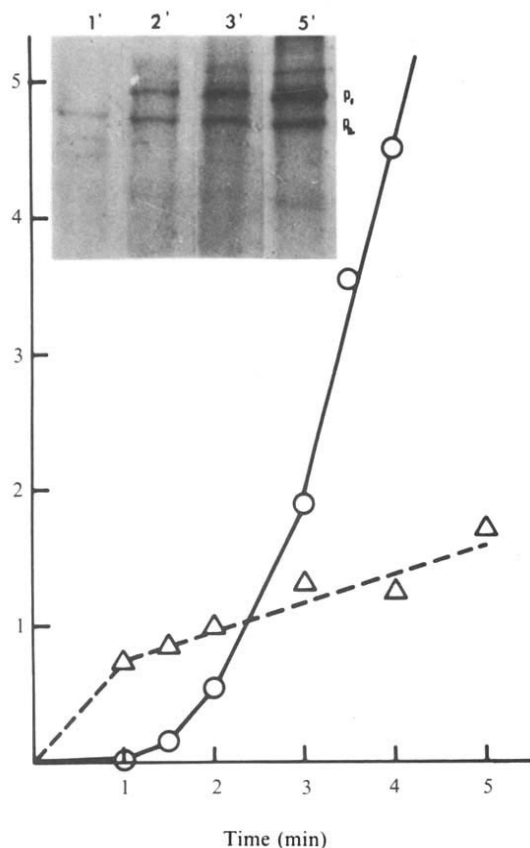


Fig. 1 Transcription of supercoiled pPS1 plasmid at low DNA/RNA polymerase ratios. Plasmid DNA was prepared as described previously^{4,20}. RNA polymerase was prepared by the method of Burgess and Jendrisak²¹. Transcription was performed in 50- μ l reactions containing 50 mM Tris-Cl (pH 8), 50 mM KCl, 10 mM MgCl₂, 10 mM 2-mercaptoethanol and 0.1 mM EDTA. DNA was added at 0.1 μ g ml⁻¹. RNA polymerase was added at 20 μ g ml⁻¹. Nucleotide concentrations were 400 μ M, the labelled nucleotide (UTP) was at 100 μ M. The reaction mixture with the polymerase was incubated at 20°C for 1 min, nucleotides were then added and the reaction was continued as shown in the figure. The reaction was stopped by the addition of an equal volume of 50 mM EDTA containing 20 μ g carrier tRNA. After ethanol precipitation the RNA was separated on gels as elsewhere⁴. Autoradiograms were scanned using a Helena Quick Scan scanner and the area of the peaks was measured using a computerized integrator and plotted against incubation time. ○, P1 activity; △, P2 activity. Two dominant bands can be seen terminating in the T1 region (see insert). The upper P1 band is about 530 bases and the lower P2 is 410 bases judging from S₁ mapping¹⁰. The two weak bands seen above the P1 band are transcripts starting at P1 and P2 that terminate at T2, the downstream terminator, giving chain lengths predicted from the DNA sequence of 688 bases and 570 bases respectively¹⁰. The weaker T2 terminated transcripts show the same effect of varying the polymerase/DNA ratio as the T1 terminated transcripts.

incorporation of UTP in the band representing transcription from P1 was fourfold greater than the incorporation in the RNA band transcribed from the P2 promoter (Fig. 1).

The relative rates of expression from P1 and P2 were found to depend on the concentration of template DNA. At 100-fold higher concentrations of DNA (10 μ g ml⁻¹) P2 serves as a much more efficient promoter than P1; the P2/P1 transcription ratio is about 2 after 4 min incubation (Fig. 2). Even at the low DNA concentration (0.1 μ g ml⁻¹), lowering the polymerase concentration 10-fold gives results similar to those in Fig. 2 (data not shown). Thus, in conditions of high enzyme to DNA ratios, P2 expression slows down when P1 expression speeds up (Fig. 1). In contrast, at low enzyme to DNA ratios, P1 and P2 expression proceeds simultaneously. Note that the total

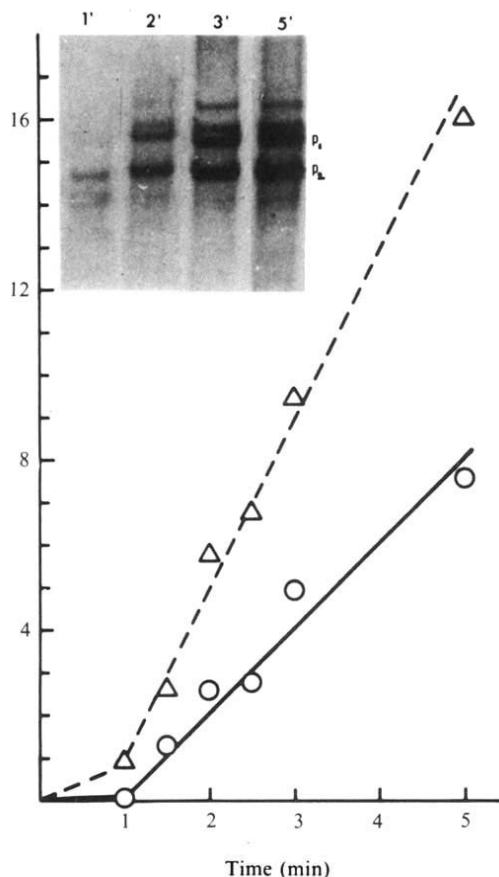


Fig. 2 Transcription of pPS1 plasmid at high DNA/RNA polymerase ratios. Transcription conditions are the same as for Fig. 1, except that DNA was added at 10 μ g ml⁻¹. The two dominant transcripts seen in the insert are transcripts from the two ribosomal promoters P1 and P2, terminating at T1. ○, P1 activity; △, P2 activity.

expression of P1 and P2 at low enzyme to DNA ratios was about one order of magnitude lower than the transcription of these promoters in high enzyme to DNA ratios (Figs 1, 2), when calculated on the basis of UTP incorporation per template (Fig. 2).

When wild-type *E. coli* cells are starved for amino acids, stable RNA synthesis is known to be inhibited when ppGpp accumulates¹². The possibility that P1 or P2 are targets for this nucleotide was studied using our *in vitro* transcription system supercoiled templates. We have observed pausing areas downstream from P2 (data not shown) similar to those described previously with linear DNA¹³. We were also able to show that all the bands that were considered as reflecting pause areas were chased to the two full-length ribosomal transcripts by cold UTP, as also noted with linear templates¹³.

The differential effect of ppGpp on the two promoters could be shown by incubating the reaction mixture in the presence of 0.3 mM ppGpp for 1 min before the addition of substrate nucleotides. Whereas transcription from P1 is inhibited 80–90% by this treatment, only 50% inhibition of P2 is observed (Fig. 3). We interpret the effect of ppGpp to be on initiation rather than on the elongation step because all the pausing areas seem to occur downstream from P2 and the effect on P1 was much greater than on P2 (see Fig. 3). Note that while GDP at the same concentrations had no effect on the transcription of these promoters, rifampicin at a concentration of 0.1 μ g ml⁻¹ showed a similar effect to that observed with ppGpp (data not shown).

Each rRNA transcription unit in *E. coli* is known to be equipped with two tandem promoters designated P1 and P2^{4–7}.

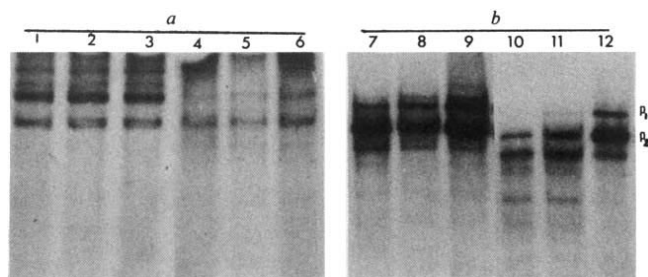


Fig. 3 The effect of ppGpp on transcription from the two tandem ribosomal RNA promoters. *a*, Transcription at low DNA/polymerase ratios, as in Fig. 1; *b*, transcription at high DNA/polymerase ratios, as in Fig. 2. Lanes 1, 2 and 3 are transcribed for 3.5, 4 and 4.5 min respectively; lanes 4, 5 and 6 are transcribed for analogous times but in the presence of 0.3 mM ppGpp. Lanes 7, 8 and 9 are transcribed for 90, 120 and 150 s, respectively; lanes 10, 11 and 12 are the analogous incubations in the presence of 0.3 mM ppGpp. 0.3 mM ppGpp was incubated with the DNA and the RNA polymerase for 1 min before initiating the transcription with the ribonucleotide substrates. Transcription conditions were as described in Fig. 1 legend.

These two promoters were found to be active *in vivo* so that in certain experimental conditions P1 was three to five times more active than P2⁷⁻¹⁰. It seems that this structure containing two promoters separated by a spacer of about 120 base pairs must have some selective advantage because this arrangement is conserved in six of the ribosomal genes sequenced so far.

We suggest that this structure may have a significant role in the control of transcription of rRNA cistrons. A low-affinity fast transcribable promoter upstream from an high-affinity, slow promoter, with a spacer between them, enables the cell to manipulate the rate of transcription in different growth conditions.

In conditions when the availability of RNA polymerase to these promoters is low, transcription would start mainly from the P2 promoter, supporting 'maintenance' ribosomal RNA synthesis. This parallels our *in vitro* conditions of a low RNA polymerase to DNA ratio, where both promoters seem to be transcribed independently, but the transcription of P2 is more efficient than the transcription of P1. In fast-growing cells, however, where RNA polymerase is abundant^{14,15}, transcription will be predominantly from P1. Due to the architecture of the promoter region, transcriptional starts from P1 could readily interfere with transcription from P2, ensuring the maximal rate of rRNA synthesis required for rapid cell growth. If this hypothesis is correct, any factor affecting the availability of RNA polymerase for the initiation of transcription will primarily affect transcription from P1. This situation parallels our experimental conditions in which the ratio of RNA polymerase to DNA is high. In these transcriptional conditions, P1 is transcribed at maximum efficiency while P2 transcription seems to be inhibited (Fig. 1). This is also in line with the phenomenon of 'promoter occlusion' described by Adhya and Gottesman¹⁶ on the expression of phage λ promoters.

Our experiments also show that factors such as ppGpp or rifampicin mainly affect transcription from P1, suggesting that this promoter is the predominant factor involved in the regulation of rRNA synthesis. These conclusions gain further support from *in vivo* experiments with pPS1 showing that during rapid growth P1 activity is about threefold more active than P2 activity and that the P1, but not P2, promoter is stringently controlled¹⁰. The high activity of P1 is also consistent with recent indications that ppGpp-sensitive promoters might bind two RNA polymerase molecules¹⁷. Note that additional factors distinct from RNA polymerase subunits may also be involved in the mechanism of *in vivo* enzyme interactions. The high efficiency of *in vitro* transcription of the rRNA promoters using supercoiled DNA is in line with experiments showing that DNA gyrase inhibitors affect ribosomal RNA cistron expression *in*

vivo^{18,19}. Thus, the use of the *in vitro* system described here in parallel with the analysis of the regulatory behaviour *in vivo* should provide a reasonable approach for understanding the determinants of control of ribosomal RNA synthesis in *E. coli*.

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Is ribonucleotide reductase the transforming function of herpes simplex virus 2?

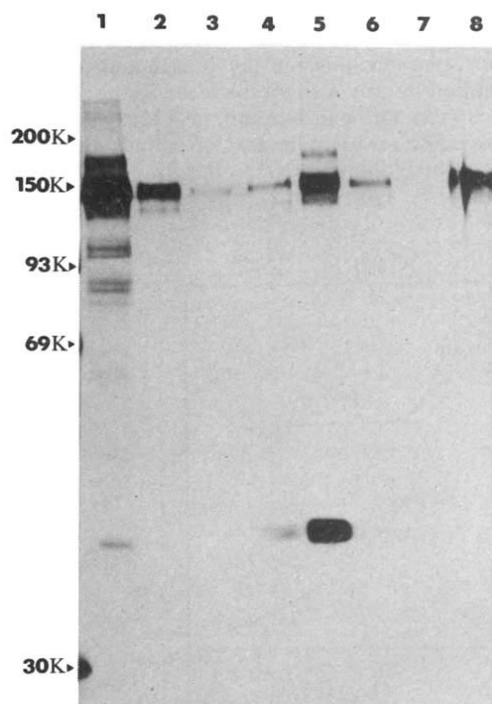
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Transformation of cells by herpes simplex virus 2 (HSV-2) can be induced by the *Bg*/II C (0.43-0.58 map units) or N (0.58-0.625) fragments of the viral genome¹⁻³. Sequences partially overlapping both fragments (0.566-0.602) encode two 3' co-terminal mRNAs⁴⁻⁶; these in turn direct the synthesis of two related polypeptides of molecular weight 140,000 (140K) and 35K (refs 4, 7), which may be involved in transformation⁷. Recently, a temperature-sensitive (ts) mutation affecting HSV-induced ribonucleotide reductase has been mapped within this common region (B. M. Dutia, personal communication). We have partially purified the induced reductase and raised a rabbit antiserum to it which inhibits the enzyme activity and immunoprecipitates from infected cells a 144K polypeptide and minor species including a 38K polypeptide⁸. Here we show that a monoclonal antibody to the putative transforming proteins⁷ competes with the rabbit serum for the 144K and 38K antigens and also immunoprecipitates specifically the induced reductase activity. These results suggest a possible role for ribonucleotide reductase in HSV-2-induced transformation.

Ribonucleotide reductase (EC 1.17.4.1) catalyses the first unique step in DNA synthesis by direct reduction of all four ribonucleotides to the corresponding deoxyribonucleotides^{9,10}. Several reports¹¹⁻¹³ have demonstrated that HSV-2, like other herpes viruses¹⁴⁻¹⁶, can induce the synthesis of a novel ribonucleotide reductase on infection of mammalian cells. The activity of the virally induced enzyme can be distinguished from that of the enzyme from uninfected cells on the basis of altered regulatory control, sedimentation properties and differential response to Mg²⁺ and salt^{8,11-13,17}. By partially purifying the

Fig. 1 Cross-reactivity between R1 rabbit antiserum to HSV-2-induced reductase and H11 monoclonal antibody. R1 serum⁸ was obtained from rabbits immunized with a partially purified preparation of viral reductase derived from HSV-2-infected cells by fractionation of crude extracts with ammonium sulphate¹³ and sedimentation of the enzymatically active salt fraction through glycerol gradients. The resulting enzyme preparation was free of cellular reductase and contained one major HSV-2 antigen of 144K and six to eight minor HSV-2 antigens, including a 38K species⁸. R1 antibodies did not react with mock-infected cells but precipitated, from HSV-2-infected cells only, a major species of 144K and a few minor species around 110K, 90K and 38K. Pre-immune rabbit serum did not recognize antigens from infected or uninfected cells⁸. H11 monoclonal antibodies raised in mice reacted only with two type-specific HSV-2 antigens of apparent molecular weights 140K and 35K⁷. For competition experiments, monolayers of BHK21 c1.13 cells were infected with HSV-2 strain 333 (ref. 26) at a multiplicity of 20 plaque-forming units per cell and labelled with ³⁵S-methionine (NEN) immediately after virus adsorption. At 7 h post-infection, when maximal reductase activity was detected¹³, the cells were collected by scraping, washed with phosphate-buffered saline and solubilized in RIPA buffer. The cell lysate was sonicated and cleared by centrifugation at 100,000g for 1 h. Immunoprecipitation was carried out by incubating 500 µl of lysate (corresponding to $\sim 3 \times 10^6$ cells and 300 µg protein) with R1 rabbit serum (50 µl) or H11 monoclonal ascitic fluid (10 µl) in the presence of 135 µl of protein A-Sepharose CL-4B beads (~ 100 mg ml⁻¹ in RIPA buffer, Pharmacia) for 2 h at 4 °C with constant mixing. The immunoprecipitates were collected by centrifugation and the supernatants reabsorbed three times with the homologous antibodies and finally with the other antibodies. The immunoprecipitates from each adsorption were then washed with RIPA buffer, solubilized in SDS-2-mercaptoethanol buffer and electrophoresed on 9% polyacrylamide-0.24% N,N'-methylene-bis-acrylamide-0.1% SDS gels. The gels were stained with Coomassie blue, destained, infused with 2,5-diphenyloxazole, dried and exposed to Kodak X-Omat film at -70 °C for 46 h. The results obtained with R1 serum are shown for the first, third and fourth adsorptions in lanes 1-3, respectively. Cross-adsorption of the final supernatant with H11 fluid is shown in lane 4. Lanes 5-8 refer, respectively, to the first, third and fourth adsorption with H11 fluid and cross-adsorption of the final supernatant with R1 serum.



HSV-2-induced reductase and raising a rabbit antiserum (R1) against it we have shown that this enzyme is also immunologically distinct from the cellular enzyme⁸. The R1 serum reacts specifically with the induced activity and has no effect on the cellular enzyme; in addition, it immunoprecipitates, solely from HSV-infected cells, a 144K polypeptide and a few minor species around 110K, 90K and 38K (ref. 8). Recent studies by B. M. Dutia (personal communication) have mapped a ts mutation which affects HSV-induced reductase activity at around 0.6 in the viral chromosome, within sequences which are known to encode at least two related polypeptides of 140K and 35K (refs 4, 7). These sequences (0.566-0.602) overlap the two fragments of HSV-2 DNA, *Bgl*II C and N, which have been implicated in transformation of cells¹⁻³. These mapping data and the similarity between the immunoprecipitation pattern obtained with the R1 serum and that reported for a monoclonal antibody (H11) to the putative transforming proteins⁷ led us to compare the two reagents for their antigen specificity and enzyme inhibitory activity.

Lysates of HSV-2-infected, radiolabelled BHK21 c1.13 cells were exhaustively adsorbed with either R1 serum or H11 ascitic fluid and finally cross-adsorbed with H11 fluid or R1 serum, respectively. The products of the reactions were analysed on SDS-polyacrylamide gels. As shown in Fig. 1, lanes 1 and 5 respectively, in our system both antibodies precipitated a 144K and a 38K polypeptide. However, R1 serum reacted preferentially with the 144K species whereas the H11 monoclonal antibodies were relatively more specific for the 38K polypeptide. In addition, the polyvalent R1 serum precipitated other minor species which were not recognized by the H11 monoclonal antibodies. Successive adsorptions with R1 serum (Fig. 1, lanes 2, 3) removed almost all the antigens recognized by H11 fluid (compare lanes 4 and 5 of Fig. 1) except for minor amounts of the 38K polypeptide. Reciprocally, H11 immunoprecipitated all the 38K (lanes 5-7) but not all of the 144K molecule, residual amounts of which could still be immunoprecipitated by the R1 serum (compare lanes 8 and 1

Table 1 Recovery of ribonucleotide reductase activity from immunoprecipitates

H11 IgG (µg)	Enzyme activity (pmol)	
	Uninfected cell enzyme	HSV-2-induced enzyme
0	34	31
50	20	367
250	16	ND
700	ND	463

Aliquots of partially purified enzyme preparations were incubated with increasing amounts of H11 IgG in the presence of protein A-Sepharose, as described in Fig. 2 legend. The immunoprecipitates were removed by centrifugation, washed twice in buffer A (20 mM HEPES pH 7.2, 1 mM DTT¹³), resuspended in buffer A containing 0.1 M DTT and incubated at 4 °C for 20 min. Samples were then brought to the reaction buffer, resulting in a final concentration of 67 mM DTT, and assayed for ribonucleotide reductase activity as outlined in Fig. 2 legend. Enzyme activity is expressed as pmol of dCMP formed in 30 min at 37 °C (ref. 13). Treatment with DTT was chosen as it is known to reduce disulphide bonds and thus should dissociate antibody molecules. Control experiments indicated that in addition this treatment inhibited the cellular enzyme by 50% and enhanced the induced activity approximately threefold. In agreement with previous data (see Fig. 2 legend and ref. 8) $\sim 30\%$ of the induced enzyme activity was found associated nonspecifically with the protein A-Sepharose beads when 350 µg of pre-immune IgG were incubated with the enzyme. ND, not determined.

of Fig. 1). We interpret these data as indicating that the two reagents have the same specificity and that their different affinities for the two polypeptides might result from differential avidities of the antibodies and/or prevalence of one type of antibody in the polyvalent R1 serum.

As previously reported⁸, R1 antibodies do not directly neutralize the HSV-2-induced reductase (a result also obtained with H11) but, in the presence of protein A-Sepharose, can specifically remove it from solution. It was therefore important

to determine whether H11 antibodies had similar properties. Figure 2 shows that incubation of R1 or H11 IgGs with the cellular reductase (open symbols), in the presence of protein A-Sepharose, resulted in only a slight decrease in the amount of enzymatic activity remaining in solution, an effect which was shown to be nonspecific as a similar loss of activity occurred when induced or cellular enzymes were incubated with pre-immune or non-immune IgGs⁸. In contrast, a significant and

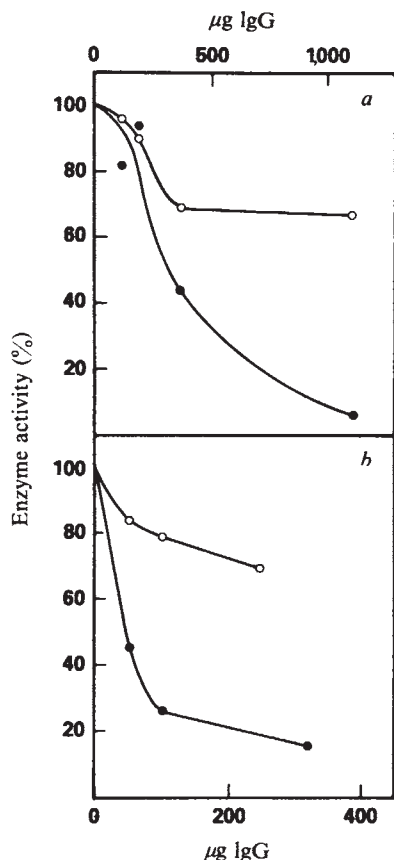


Fig. 2 Effect of IgG purified from R1 serum or H11 ascitic fluid on ribonucleotide reductase activity. Reductase activity is expressed as a percentage of the activity measured after incubation of the enzyme samples with protein A-Sepharose beads in the absence of IgG (100%). Closed symbols refer to activity of the HSV-2-induced enzyme, open symbols to activity of the uninfected cell enzyme after incubation with R1 IgG (a) or H11 IgG (b). As reported⁸, incubation of either enzyme with pre-immune IgG resulted in a limited inhibition of activity (a maximum of 35% with 350 µg of pre-immune IgG), the extent and kinetics of which parallel the inhibition obtained on incubation of the uninfected cell enzyme with immune IgG (open symbols). This limited inhibition appears to be nonspecific as it was also observed on incubation of the enzyme with protein A-Sepharose beads in the absence of antibodies.

Methods: IgG was purified from R1 serum or H11 ascitic fluid by adsorption to columns of protein A-Sepharose CL-4B beads followed by elution with 0.1 M glycine, pH 2.5 (ref. 8). The IgG fraction was then brought to neutral pH, dialysed and concentrated to 10–25 mg ml⁻¹. Ribonucleotide reductase was partially purified from HSV-2-infected or mock-infected BHK 21 cl.13 cells by fractionation of cell extracts with ammonium sulphate, as described elsewhere¹³. This procedure provides a virus-induced enzyme preparation essentially free of cross-contaminating cellular reductase. Aliquots of the enzymes were mixed with increasing amounts of R1 or H11 IgG in the presence of 6 mg of protein A-Sepharose beads, in a total volume of 100 µl. After incubation at 4 °C for 1.5 h under constant mixing, immunoprecipitates were removed by centrifugation and the supernatants assayed for residual enzymatic activity by monitoring the reduction of CDP¹³. Briefly, supernatants were brought to the reaction buffer, incubated at 37 °C for 30 min and aliquots of the reaction analysed by descending paper chromatography after acid hydrolysis and neutralization of the samples.

specific decrease in activity was obtained when the induced reductase (Fig. 2, closed symbols) was incubated with increasing amounts of R1 or H11 IgGs. Indeed, in this respect the H11 antibodies (Fig. 2b) were much more efficient, possibly because of their monoclonal nature. Table 1 shows the results of an experiment designed to recover enzymatic activity from immunoprecipitates. Preliminary experiments indicated that the immunoprecipitates were inactive when assayed directly (not shown), but that reductase activity could be recovered from them on incubation with 0.1 M dithiothreitol (DTT). A direct correlation was established between the amounts of induced activity recovered and the amounts of IgG used for immunoprecipitation; no such correlation, and indeed no significant activity, was obtained for the cellular enzyme. Thus, H11 antibodies, which are specific for the same polypeptides (Fig. 1) and enzymatic activity (Fig. 2) as the R1 antibodies, are able to immunoprecipitate the HSV-2-induced reductase (Table 1).

Together with the results of others (refs 1–3 and B. M. Dutia, personal communication), these data suggest that HSV-2 DNA sequences which have been implicated in transformation of mammalian cells might encode the viral ribonucleotide reductase. Which of the two proteins encoded by this region is responsible for enzymatic activity and/or transformation has not yet been determined and in fact both could be involved^{1,3}. Transformation of cells by HSV-2 seems to differ from transformation by other DNA viruses in that viral transforming sequences need not be retained by the cells for maintenance of the transformed phenotype^{3,7,18,19}. This observation supports the hypothesis that HSV-2 might transform via a mutagenic 'hit-and-run' mechanism which requires only transient expression of viral genes^{20,21}. Such a mutagenic effect could be exerted by the transient expression of the viral reductase. In both prokaryotes and eukaryotes this enzyme tightly controls the levels of deoxyribonucleotide pools by the coordinated reduction of all four ribonucleotides^{9,10}. Such control is essential for maintaining the fidelity of DNA replication because imbalances in the deoxyribonucleotide pools (which may cause base substitutions, altered proofreading or error-prone repair) are associated with a mutator phenotype^{22,23}. Indeed, ribonucleotide reductases altered in their regulatory control can act as mutators²³. The enzyme induced by HSV-2 is quite distinct from the cellular enzyme in that it does not respond to feedback inhibition by dTTP or dATP^{11–13}, and in fact HSV infection of cells has been shown to induce imbalances in the levels of deoxyribonucleotides^{24,25}. The model involving the HSV reductase in cell transformation predicts that infection of cells with virus, or transfection with viral sequences coding for the enzyme, would be mutagenic. This prediction is subject to verification.

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Note added in proof: We have recently produced a neutralizing antibody specific for the viral reductase and the 144 K and 38 K HSV-2 antigens.

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Characterization of a human colon/lung carcinoma oncogene

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DNA sequences capable of inducing oncogenic transformation of NIH3T3 mouse cells are found in a number of human tumour cell lines. When DNAs of these cell lines are applied to monolayer cultures of the mouse fibroblasts, foci of transformed cells are observed 2-3 weeks later¹⁻⁵. DNA from cells of such primary foci can be used in turn to induce foci in a second cycle of gene transfer^{2,3}. The human DNA sequences responsible for transformation have been called oncogenes, the best characterized of which is closely related to the Harvey murine sarcoma virus oncogene. Here we present a characterization of an oncogene which we found originally to be present in DNA of the SW480 colon carcinoma cell line. We indicate its structural outlines and demonstrate, in extension of reported results^{3,4}, its presence in an activated form in the genome of several types of human tumour cell lines as well as in biopsy tissue from an adenocarcinoma of the large bowel. We identify this tumour oncogene with c-Ki-ras2, one of two known members of the Kirsten ras family of human proto-oncogenes, extending a series of recent reports⁵⁻⁷ which have demonstrated homologies between human oncogenes and those of Harvey and Kirsten murine sarcoma viruses. The c-Ki-ras2 oncogene of several tumour cell lines is shown to be amplified.

The close association of *Alu* segments with a transfected oncogene provides one means of determining a structural outline of the transforming region. In Fig. 1, lanes *a*, *b*, we show the pattern of *Alu*-containing, *Eco*RI segments found in the DNAs of secondary foci induced by serial passage of the oncogene of the SW480 human adenocarcinoma cell line. As reported previously³, these seven segments of 2.8-6.7 kilobases (kb) in size are seen in all secondary foci induced by the passage of DNAs from three independent primary transformants. We find these seven segments conserved as well in nine tertiary transformants induced by yet another round of serial passage of the oncogene (data not shown). Stated differently, the association of these seven fragments with the oncogene has survived 24 transfection events in this laboratory, as well as in 51 transfection events in experiments reported by others^{4,5}. It is thus most likely that conserved fragments contain essential elements of the oncogene. We have summed the sizes of the

conserved oncogene segments generated by endonuclease *Eco*RI, as well as by six other endonucleases (*Bam*HI, *Hind*III, *Bgl*II, *Sac*I, *Kpn*I, *Pvu*II). These calculations all indicate that the oncogene is between 30 and 35 kb in length.

Others have recently reported that transfectants of a Lx-1 lung carcinoma have acquired a novel 3-kb *Eco*RI DNA fragment reactive with sequence probe derived from the *ras* oncogene of Kirsten murine sarcoma virus⁵. We have confirmed this finding and related it to the present work. As seen in Fig. 1c, *d*, transfectants acquiring the colon oncogene exhibit three novel *Eco*RI segments reactive with the v-Ki-ras probe. In addition to the reported 3-kb segment, we find a second, closely migrating segment of approximately 3.2 kb and a third segment of 6.7 kb. The 3.2-kb and 6.7-kb fragments correspond precisely with two of the oncogene segments previously detected with the human *Alu* sequence probe (data not shown). The 3.0-kb segment is free of these repeated sequences and consequently was not detected by the *Alu* probe. Experiments to be described elsewhere confirm the presence of a novel protein of molecular weight 21,000 in these transfectants compared with their untransfected NIH3T3 counterparts. This protein exhibits specific immunological reactivity with monoclonal antisera raised against the *ras* gene products. Of some interest is the fact that the p21 proteins induced by the colon and lung oncogene exhibit altered electrophoretic mobilities (E. M. Scolnick and R.A.W., in preparation). This finding mirrors the altered behaviour of p21 *ras* protein in bladder carcinoma transfectants whose oncogene carries a lesion in a protein-encoding region⁸.

Previous results have suggested the existence of two separate Ki-ras homologues in the human genome, termed c-Ki-ras1 and c-Ki-ras2⁹. Clones of portions of these two oncogenes have been obtained by screening a genomic library of normal human DNA (provided by T. Maniatis) using as probe a clone of the Kirsten sarcoma virus genome⁹. We wished to determine the relationship between the tumour oncogene and these two human Ki-ras homologues. Figure 1 shows that a *Bgl*II endonuclease fragment of 4.5 kb is detectable by the viral oncogene probe in normal human DNA (lane *g*), and in the phage containing much if not all of the Ki-ras1 human gene (lane *h*). This 4.5-kb *Bgl*II segment is absent from the mouse transfectant carrying the colon/lung oncogene (Fig. 1, lane *i*). Having found a negative correlation between c-Ki-ras1 sequences and the oncogene, we attempted to demonstrate identity between the oncogene and c-Ki-ras2. To do this, a segment of the c-Ki-ras2 bacteriophage⁹ was obtained by preparative gel electrophoresis and used as sequence probe in the blot-transfer shown in Fig. 1, lanes *k*, *l* and *m*. This sequence probe was specific for the c-Ki-ras2 gene since it contained neither *Alu* sequences nor protein-encoding sequences of the gene. (The presence of such coding sequences in the probe might cause the probe to be cross-reactive with segments of the c-Ki-ras1 gene.) The specificity of this probe is further supported by its failure to detect the 4.5-kb *Bgl*II fragment which contains the bulk of the c-Ki-ras1 gene (Fig. 1, lane *m*).

Figure 1 lanes *l* and *m* shows that this c-Ki-ras2 probe detects a 25-kb *Bgl*II segment in human DNA which is present as well in the transfectant. Taken together with the previous data, this result suggests that the colon/lung oncogene is directly related to the sequences previously defined as c-Ki-ras2. Moreover, the segregation of the two c-Ki-ras genes from one another, which occurred as a consequence of transfection, provides the first direct evidence that these genes constitute two separable and distinct genetic entities. An active form of the human c-Ki-ras1 gene has not yet been demonstrated.

The affiliation of the 30-35 kb-colon/lung oncogene with the *ras* gene family yields a marked contrast with another, better-characterized *ras* gene, the cHa-ras1 oncogene, activated alleles of which are the EJ and T24 bladder carcinoma oncogenes which have recently been isolated as molecular clones¹⁰⁻¹². The Ki-ras and Ha-ras genes encode 21,000 daltons of almost indistinguishable sequence (>90% identity)^{13,14}. The

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Fig. 1 Southern blot analysis of SW480 transfectants. A sample of 10 μ g of each DNA was digested with endonuclease *Eco*RI (lanes *a-f*) or with endonuclease *Bgl*II (lanes *g-m*), fractionated by electrophoresis through a 1% agarose gel and transferred to nitrocellulose paper¹⁹. The filters were incubated with 5×10^6 c.p.m. of nick-translated 32 P-labelled probe. The probe used in lanes *a* and *b* was BLUR8. In lanes *c-j*, the probe was the v-Ki-ras probe HiHi3²⁰. In lanes *k-m* the probe was a 0.5 kb *Eco*RI-*Xba*I fragment isolated from the c-Ki-ras2 bacteriophage⁹. The DNAs analysed in lanes *a-d* were from the cell lines of the following secondary foci induced by passages of the oncogene of the SW480 colon carcinoma cell line. *a*, SW-3-1; *b*, SW2-3; *c*, SW2-1; *d*, SW-1-1; *e*, NIH3T3, mouse DNA; *f*, Lx-1, a human small cell carcinoma cell line; *g*, human fetal brain DNA; *h*, NIH3T3 to which 50 pg of the c-Ki-ras 1 bacteriophage was added; *i*, SW2-1; *j*, NIH3T3; *k*, NIH3T3; *l*, SW2-1; *m*, human fetal brain DNA. The size markers indicated were λ phage DNA digested with the endonuclease *Hind*III.

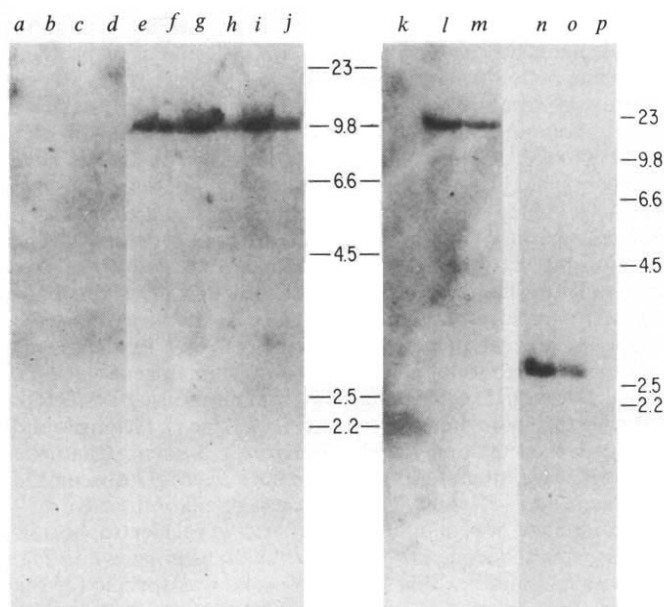
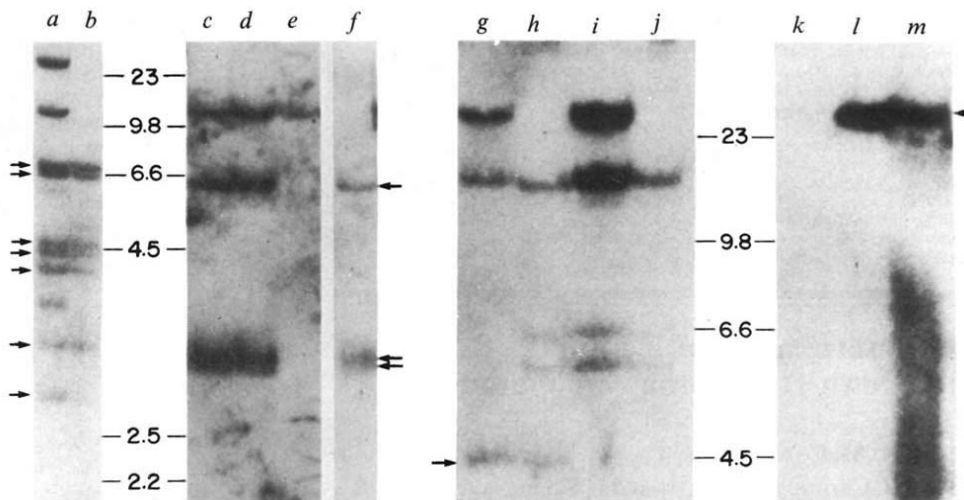


Fig. 2 Southern blot analysis of digested cellular DNAs from various transfectants probed with the oncogene specific probe p640. Samples of 10 μ g of each DNA were digested with endonuclease *Pvu*II (lanes *a-j*), *Xba*I (lanes *k-m*), or *Eco*RI (lanes *n-p*). Gel electrophoresis, transfer and preparation of probe were as described in Fig. 1. The transfer filters were incubated with 5×10^6 c.p.m. nick translated 32 P-labelled p640. The DNAs analysed are from the following cell lines. *a*, NIH3T3; *b*, SH1-1, a cell line derived from a secondary focus induced by DNA from the human neuroblastoma cell line SK-N-SH; *c*, EJ6-2, a cell line derived from a secondary focus induced by DNA from the human bladder cell line EJ; *d*, HL60-1-9, a cell line derived from a secondary focus induced by DNA from the human promyelocytic leukaemia cell line HL-60; (lanes *a-e* are from a separate part of the same filter as lanes *e-j*); *e-i*, A5-6, A5-7, A5-8, A5-9, A5-10, cell lines derived from primary foci induced by DNA from the human adenocarcinoma cell line A549; *j*, SW-3-2, a cell line derived from a secondary focus induced by DNA from the human colon carcinoma line SW480; *k*, NIH3T3; *l* and *m*, Lx1-1, Lx1-2, cell lines derived from primary foci induced by DNA from the human small cell carcinoma cell line Lx1; *n*, Duke 2-16, a cell line derived from a secondary focus induced by DNA isolated from a fresh tumour of the large bowel; *o*, SW2-3, a cell line derived from a secondary focus induced by DNA from the human colon carcinoma line SW480; *p*, NIH3T3.

c-Ha-ras1 gene is <6.6 kb in size while the related c-Ki-ras2 is 5-6 times larger. Only 2% of the c-Ki-ras2 appears to be devoted to encoding amino acid sequence.

We attempted isolation of a sequence probe specific for the SW480 oncogene, by exploiting the association of the *Alu* sequences with the oncogene. DNA of a 2° transformant was cleaved to completion with the endonuclease *Eco*RI and the resulting fragments introduced into the λ phage vector Charon 16A. This λ phage library was then screened for human segments by the Benton-Davis hybridization technique¹⁵ using an *Alu* sequence probe, termed BLUR8 by its originators¹⁶. This approach allowed the isolation of a bacteriophage carrying 2.8 kb *Eco*RI fragment that is reactive with the *Alu* probe.

This cloned 2.8-kb fragment segment, when used as sequence probe, preferentially reacts with only one of the seven segments of the oncogene previously detected by the *Alu*-specific probe (data not shown). The 2.8-kb segment present in this λ phage and the uncloned genomic counterpart detected by *Alu*-sequence blotting behaved identically upon further treatment with eight other endonucleases (data not shown). Since sequences reactive with this 2.8 kb probe are seen in all SW480 transformants, it must derive from a DNA segment intimately associated with the oncogene. A 640-bp *Eco*RI-*Hind*III fragment was prepared from the 2.8-kb λ phage insert. This smaller segment, which lacks *Alu* sequences, was propagated in the plasmid vector pBR322. The resulting chimaeric plasmid DNA, termed p640, served as a specific probe for the oncogene.

This SW480 oncogene probe was used to measure relatedness of various transforming sequences present in other types of tumour cell DNAs. Specifically, we wished to ascertain whether a mouse cell transformed by human tumour DNA exhibited a novel DNA segment homologous to the p640 colon oncogene probe. As seen in Fig. 2, the DNAs of NIH3T3 cells transformed by DNAs from the EJ human bladder carcinoma cell line, the SK-N-SH human neuroblastoma cell line, and the HL60 human promyelocytic leukaemia cell line, exhibited no reactivity with the 640 nucleotide probe. In contrast, sequences reactive with this probe are present in NIH3T3 cells transformed by DNA from the human lung adenocarcinoma cell line A549 or from the small cell lung carcinoma cell line Lx-1. The origin of these Lx-1 cells is supported by the presence in their karyotype of a chromosome 3 deletion characteristic of small cell carcinomas (ref. 17 and S. Latt, personal communication). These results argue strongly that these two dissimilar types of lung carcinoma cells carried active transforming genes which are homologous to the colon carcinoma oncogene probe. This supports work of

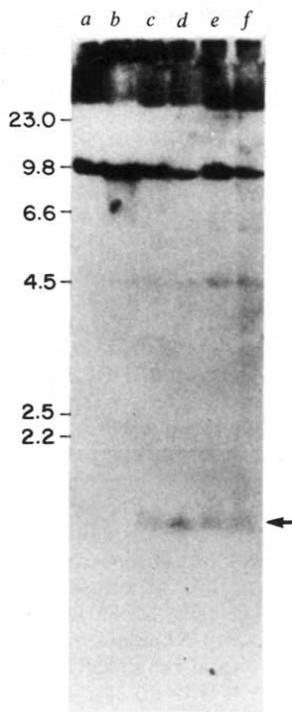


Fig. 3 Southern blot analysis of cellular DNAs digested with *PvuII*. Samples of 10 µg DNA were digested, fractionated by electrophoresis and transferred as described in Fig. 1. The filter was probed with 5×10^6 c.p.m. each of ^{32}P -labelled p640 and ^{32}P -labelled human insulin intervening sequence probe. DNAs analysed are from the following cell lines. *a*, *b*, SW-3 and SW-2, two cell lines derived from primary foci induced by chromatin isolated from the SW480 cell line; *c*, SW480; *d*, human fetal brain DNA; *e*, Lx1; *f*, A549.

others who have found that lung and colon carcinoma transformants exhibits similar patterns of human *Alu*-containing DNA fragments⁴.

DNAs of six independent human tumour cell lines have yielded transforming activity that can be identified with this oncogene^{3,4} (Fig. 2). It might be argued, however, that these various oncogenes have arisen during the establishment of the respective tumour cell lines and do not reflect the existence of actively oncogenic sequences present in the tumours from which these cell lines were derived. We therefore attempted to detect this oncogene in a series of DNAs extracted directly from tumour biopsies. As indicated in Fig. 2, lane *n*, DNA obtained from resection of an adenocarcinoma of the large bowel was also able to induce foci, which carried copies of the colon/lung oncogene detectable using the p640 probe. We conclude that a version of this oncogene existed in an active state in this tumour before any *in vitro* manipulations of the tumour cells.

The availability of the p640 and HiHi3-Ki-ras probes made it possible to compare the physical map of the transfected oncogene with related sequences present in the normal human genome. By using either of these probes in the comparative analysis of oncogene and normal human DNA cleaved with any one of five endonucleases, we have been unable to resolve any differences in the physical maps of the oncogene and the related human DNA sequences (data not shown). We conclude that the oncogene is derived from a related normal sequence, which can be termed a proto-oncogene, and that the steps leading to the activation of the proto-oncogene have not involved gross physical rearrangement of its sequences.

We used the p640 probe to examine the copy number of the oncogene in DNA of normal human cells and in DNA of the tumour cell lines SW480, A549 and Lx-1. In order to do this, we required an accurate measure of the DNA concentration in each sample. We measured DNA concentrations in two ways.

First, DNA concentrations were measured in a fluorescent spectrophotometer which specifically detects DNA fluorescence. Second, an additional probe for a single copy human gene was included with the p640 colon oncogene probe as an internal control of DNA concentration. As seen in Fig. 3, the p640 probe detects a *PvuII* endonuclease fragment of 9.8 kb in normal human fetal DNA, donor tumour cell DNAs, and transfectant DNAs. A 1.5-kb band detected by a human insulin intervening sequence probe (Fig. 3, arrow) is of comparable intensity in the four human DNA channels. Comparison of the fetal DNA (lane *d*) with DNAs of the donor SW480 colon carcinoma and Lx-1 small cell carcinoma cell lines (lanes *c*, *e*) indicates that the oncogene is amplified in the tumour cell DNAs. By varying the radioautographic exposure times, we estimate that the SW480 oncogene is amplified by a factor of 5. That of the Lx-1 cell line is amplified by a factor of 3. It does not appear that the SW480 oncogene is substantially amplified in the A549 cell line (lane *f*). However, we cannot rule out a low level of amplification of this gene in this cell line. These data also indicate that the oncogene is present in multiple copies in the two lines transfected with chromatin from the SW480 colon carcinoma cell line (Fig. 3, lanes *a*, *b*). This was consistent with previous work which showed that donor markers become established in multiple copies within recipient cells³.

This direct demonstration of amplification of the c-Ki-ras2 gene is to be contrasted with indirect indications presently available for a second, independent alteration of the gene. This postulated second alteration is a mutation which imparts transforming activity to individual copies of the gene. Although the c-Ki-ras2 gene is present in multiple copies in some tumour cells, the procedure of transfection allows introduction of at most one copy of this gene into a given recipient cell. [This stems from the fact that a recipient only stabilizes approximately 10^{-3} of a donor cell genome¹⁸.] Consequently, the transformation of a transfected cell derived from acquisition of only a single copy of a c-Ki-ras2 tumour gene. The comparable c-Ki-ras2 allele of normal cells has never induced foci in our hands, and we conclude that the version of this gene present in donor tumour cells is structurally different from that version present in normal tissues. Elucidation of this structural alteration will reveal the nature of the second event which, together with the gene amplification, contributed to creating the tumours represented by the SW480 and Lx-1 cell lines.

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Comparison of transformation efficiency of human active and inactive X-chromosomal DNA

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The mechanism of X-chromosome inactivation has been investigated recently using DNA-mediated transformation of the X-linked hypoxanthine phosphoribosyl transferase (*hprt*) locus. Several experiments indicate that inactive X-chromosomal DNA does not function in HPRT transformation. Liskay and Evans¹ used DNA from hamster or mouse cells which had an *hprt*⁻ allele on the active X chromosome and an *hprt*⁺ allele on the inactive X chromosome. We and others^{2,3} used rodent-human hybrid cell lines which had an *hprt*⁺ allele on the inactive human X chromosome alone. DNA from all of these cells failed to transform HPRT⁻ recipients. Recently, Chapman *et al.*⁴ have shown that inactive X-chromosome DNA from several tissues of adult female mice is strikingly inefficient in genetic transformation for the *hprt* gene. On the other hand, de Jonge *et al.*⁵, using simian virus 40 (SV40)-transformed fibroblasts from a human heterozygous for an HPRT deficiency, observed HPRT transformation regardless of whether the *hprt*⁺ allele was on the active or the inactive X chromosome of the donor cells. We have done an experiment similar to that of de Jonge *et al.*⁵, and report here results which clearly indicate that DNA from the inactive X chromosome functions very poorly in HPRT transformation, thus supporting the original interpretation of Liskay and Evans¹ that inactive X-chromosomal DNA is structurally modified.

The fibroblast cell line, 405, used as the source of DNA in our experiments, was derived from the heterozygous mother of a Lesch-Nyhan patient (HPRT-deficient). Hair follicle studies on the mother and ³H-hypoxanthine autoradiographical studies on her cell culture demonstrated two populations of

cells in both tissues⁶, as expected from an individual heterozygous at an X-chromosome locus. The fibroblasts were split into two populations and expanded under different selection regimens. Strain 405H was grown in hypoxanthine-aminopterin-thymidine (HAT) medium⁷ yielding cells having the *hprt*⁺ allele on the active X chromosome (HPRT⁺), while strain 405TG was grown in 6-thioguanine (6-TG) medium⁸, yielding cells with the *hprt*⁺ allele on the inactive X-chromosome (HPRT⁻). Before DNA isolation, the cells were tested for the appropriate phenotype by HPRT assay⁹ and growth in the opposite selective medium. Strain 405H exhibited normal HPRT activity (2.5×10^2 pmol per h per 10^6 cells) and did not grow in 6-TG medium, while 405TG had no detectable HPRT activity and did not grow in HAT medium. The cells were also checked for sex chromatin (Fig. 1) and both lines were positive, indicating the presence of an inactive X chromosome.

Four separate transformation experiments were performed and are summarized in Table 1. For DNA from 405TG cells, we found a single unstable transformant in a total of 9.2×10^7 cells. The same DNA preparation gave a normal transformation rate for thymidine kinase (TK), an autosomal marker (24 clones out of a total of 4.2×10^7 cells). DNA from 405H cells gave a transformation frequency of 29 clones from a total of 6.0×10^7 cells, which is in the normal range for HPRT transformation in our laboratory for cells with an *hprt*⁺ allele on the active X chromosome. We analysed over 50 transformants produced by DNA from cells having an *hprt*⁺ allele on the active X chromosome, and all these clones have been demonstrated as transformants by electrophoretic analysis.

deJonge *et al.*⁵ used SV40-transformed cells as their source of DNA, which could account for the difference between their results and ours. To explore this possibility, we carried out an experiment using an SV40-transformed 405TG culture. At the second passage in 6-TG medium, 1×10^6 405TG cells were treated with 10^7 plaque-forming units (PFU) of SV40 strain 776. The resulting line, 405SVTG, was grown and tested as described for 405TG, and showed sex chromatin, but no HPRT activity before DNA isolation. In three experiments using DNA from 405SVTG cells, we found 3 apparent transformants out of a total of 8.4×10^7 cells. One clone had no detectable ¹⁴C-guanine incorporation (see Table 1) and exhibited no activity

Table 1 Transformation of 613 and LTK with 405H, 405TG and 405SVTG DNA

Experiment	DNA donor	613 Selection for HPRT ⁺			LTK Selection for TK ⁺		
		Positive/total plates	Colonies/cells treated	Transfer frequency	Positive/total plates	Colonies/cells treated	Transfer frequency
1	405TG	0/8	0/16 × 10 ⁶	0	4/5	6/10 × 10 ⁶	6.0 × 10 ⁻⁷
2	405TG	0/15	0/30 × 10 ⁶	0	5/5	7/10 × 10 ⁶	7.0 × 10 ⁻⁷
	405SVTG	0/15	0/30 × 10 ⁶	0	5/5	8/10 × 10 ⁶	8.0 × 10 ⁻⁷
	405H	10/10	13/20 × 10 ⁶	6.5 × 10 ⁻⁷	—	—	—
3	405TG	1/18	1/36 × 10 ⁶	2.8 × 10 ⁻⁸	11/11	11/22 × 10 ⁶	5.0 × 10 ⁻⁷
	405SVTG	0/1	0/2 × 10 ⁶	0	5/5	6/10 × 10 ⁶	6.0 × 10 ⁻⁷
	405H	13/17	13/34 × 10 ⁶	3.8 × 10 ⁻⁷	—	—	—
4	405TG	0/5	0/10 × 10 ⁶	0	—	—	—
	405SVTG	1/26	2/52 × 10 ⁶	3.8 × 10 ⁻⁸	—	—	—
	405H	2/3	3/6 × 10 ⁶	5.0 × 10 ⁻⁷	—	—	—

The HPRT⁻ cell line, 613, was derived from the LTK⁻ line² by DeMars and is apparently nonreverting³. DNA was isolated from the donors by lysing the cells directly in their flasks and treating it as described previously². The DNA solutions used had an A_{260}/A_{280} of 1.7–1.8. DNA lengths were shown to be >50 kilobase pairs on 0.4% agarose gels. The transformation procedures have been previously described². In experiment 1, a procedure involving a 30-min dimethyl sulphoxide exposure was performed². In experiments 2 and 3, the modification of Corsaro and Pearson was used¹⁶. Each plate contained $\sim 2 \times 10^6$ cells at the time when 20 μ g of DNA were added. Selective media (613, hypoxanthine-azaserine¹⁷; LTK⁻, HAT) were changed every 4 days and clones appeared after about 2 weeks. Plates without clones were fed for 4 weeks, then stained with 50% methanol/1% Coomassie brilliant blue R to confirm the absence of clones. The medium used for human cell expansion was Eagle's minimal essential medium (MEM; flow) plus 15% fetal bovine serum and gentamicin. The cells were grown in an atmosphere of 2% O₂, 5% CO₂ and 93% N₂. Cells for transformations were grown in Ham's F12 media with 10% fetal bovine serum and gentamicin in an atmosphere of 5% CO₂. ¹⁴C-guanine incorporation was determined by adding 1.0 μ Ci of ¹⁴C-guanine (56.7 mCi mmol⁻¹; NEN) to 1×10^5 cells in 3 ml of MEM. Incubation was continued for 4 h, followed by removal of media and washing in phosphate-buffered saline and methanol. After air drying, 1-cm plugs were cut from the cell surface and counted in Omnifluor in a liquid scintillation counter. In these conditions, known HPRT⁻ cell lines had background counts, while normal HPRT⁺ cultures averaged over 8,000 c.p.m.

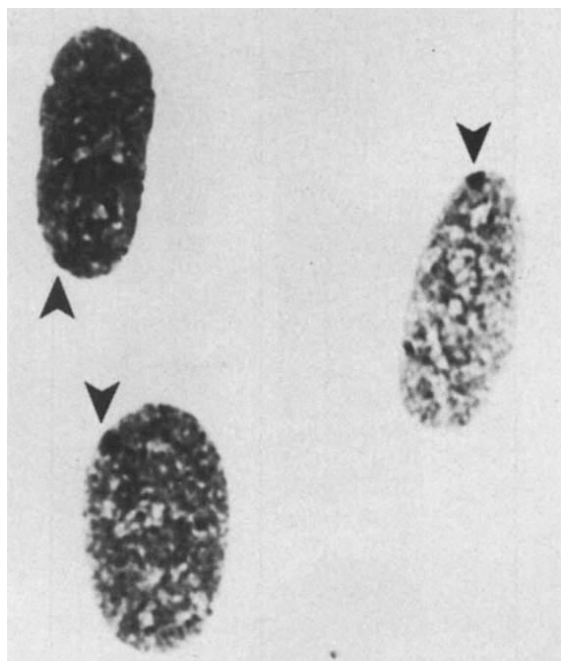


Fig. 1 Barr body (arrowheads) in 405TG cells. Cells were grown on sterile coverslips and fixed in methanol/acetic acid (3:1) for 20 min. After hydrolysis in 5 M HCl at room temperature for 15 min, followed by rinsing with water, the cells were stained with Tetrachrome (Sigma; 1.5 mg ml⁻¹ methanol combined with 2 vol. Gurr's phosphate buffer, pH 6.8) for 5 min, then rinsed with water.

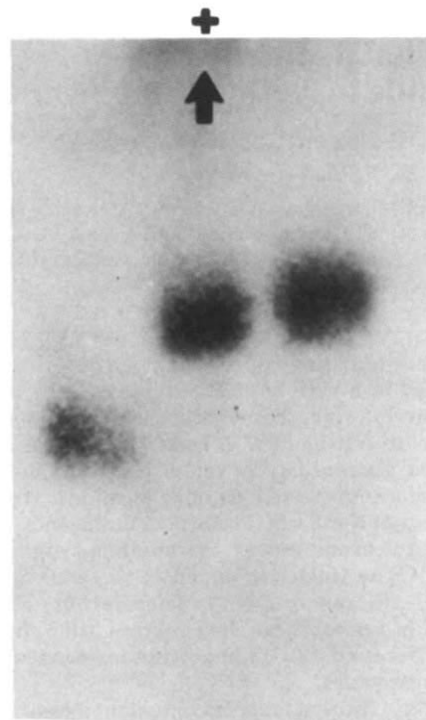


Fig. 2 Starch gel electrophoresis of the HPRT enzyme². Lane 1 is a normal mouse fibroblast extract. Lane 2 is a transformant produced by 405SVTG DNA, and lane 3 is a normal human fibroblast extract.

after electrophoresis. This clone was clearly not a transformant; it may have been azaserine-resistant. Another clone had normal ¹⁴C-guanine incorporation and was shown to be a transformant by electrophoretic analysis (Fig. 2). The third clone was detected by staining and therefore not available for ¹⁴C-guanine incorporation or electrophoretic analysis. Thus, we found a maximum of two transformants out of 8.4×10^7 cells tested, which is 20-fold lower than the HPRT 405H DNA transformation frequency. As with 405TG DNA, the 405SVTG DNA gave a normal TK transformation frequency.

It is not apparent why the results of deJonge *et al.*⁵ are so different from all the other transformation experiments involving inactive versus active X-chromosome DNA. One possible explanation is that deJonge *et al.*⁵ used a single SV40-transformed clone for each donor cell line, while we used a mass-transformed culture. It may be that they have studied a novel event which will be difficult to examine experimentally. SV40 has been shown to cause deletions¹⁰, and it has interesting gene promoter activity¹¹, but it is not obvious how it could affect a cell such that the *hprt* gene was activated only on transformation. Another explanation of the deJonge *et al.*⁵ result is that they did not expand their cells under selection as we did, and it is possible that a subpopulation of their HPRT⁻ line became contaminated with HPRT⁺ cells. Finally, it is possible that the HPRT mutation used by deJonge *et al.*⁵ is functional in HPRT transformation. For example, if the mutation were in a controlling site at some distance from the structural gene, it is possible that this region could become separated from the structural gene during transformation, allowing expression of the gene. This last consideration could apply also to our work as an explanation of the few transformants observed when the *hprt*⁻ allele was on the active X chromosome. However, a preliminary study of 35 plates treated with DNA from cells of the Lesch-Nyhan son of our heterozygote gave no transformants.

The failure of inactive X-chromosomal DNA to function in HPRT transformation has been interpreted to mean that the inactive X-chromosomal DNA is modified, possibly by methylation of cytosine residues. Experiments with 5-azacytidine, a

cytosine analogue which cannot be methylated and therefore causes demethylation of DNA, support this idea¹². This analogue has been shown to reactivate genes located on human inactive X chromosomes in mouse-human hybrid cell lines^{3,13}. This reactivated X-chromosomal DNA is capable of transformation of HPRT⁻ cells to an HPRT⁺ phenotype^{2,3}, suggesting that its DNA structure has been changed by the 5-azacytidine treatment, most probably by demethylation. Furthermore, *in vitro* methylation of cloned DNA inhibits its ability to transform, while those transformants which do arise carry partially demethylated DNA^{14,15}. In conclusion, our report that human inactive X-chromosomal DNA functions at a very low level in transforming HPRT⁻ mouse cells to an HPRT⁺ phenotype provides additional evidence for the hypothesis that the molecular basis of X inactivation involves a change at the DNA level.

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Is there left-handed DNA at the ends of yeast chromosomes?

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Tracts of the alternating copolymer poly(dGdT·dCdA) have been observed in a variety of eukaryotes¹. Such tracts are of particular interest since homopolymers of this sequence can exist *in vitro* as left-handed Z form DNA². We have found that the yeast *Saccharomyces cerevisiae* contains at least 30 poly(GT) tracts at dispersed genomic locations. We show here that one subset of these tracts is located at the ends (telomeres) of the yeast chromosome. In addition, we show that poly(dGdT·dCdA) tracts are added to the ends of the extra-chromosomal ribosomal DNA molecules of *Tetrahymena* when cloned in yeast. These data represent the first reported association between a homopolymeric sequence and a chromosome structure.

Miesfeld *et al.*¹ showed that a recombinant plasmid containing a tract of (GT)₁₇ residues hybridized to DNA isolated from mouse, human, pigeon, *Xenopus*, slime mould and yeast. Hamada and Kakunaga³ used a synthetic poly(dGdT·dCdA) hybridization probe to show that (GT)_n tracts were widely distributed in the human genome. In order to examine the number of such tracts in the yeast *S. cerevisiae* genome, we labelled poly(dGdT·dCdA) by nick translation and used this hybridization probe to examine *MspI*-derived restriction fragments of yeast nuclear DNA. By Southern blot analysis⁴, we detected at least 30 bands of hybridization (Fig. 1). The same pattern of hybridization was also observed when a plasmid¹ containing human DNA with a (GT)₁₇ tract was used as a hybridization probe (data not shown). In different strains, we frequently observed different patterns of hybridization to the poly(dGdT·dCdA) probe. For example, the haploid strain A364a contained about five bands that did not co-migrate with bands derived from the DNA of the haploid strain 2262. We crossed these haploids together to form a diploid (+D4) that was then put through meiosis. Five tetrads were dissected and cultures were grown from each spore. DNA was isolated, treated with *MspI* and examined by Southern analysis. In each tetrad examined, bands of hybridization that were different in A364a and 2262 segregated 2 to 2 (two spores had bands and two did not). This type of segregation (Fig. 1a) strongly suggests that some of the GT tracts are located on centromere-containing nuclear chromosomes. Since the DNA for these studies was isolated using a density gradient procedure that removes mitochondrial DNA⁵, we believe that all the observed GT tracts occur within nuclear DNA.

Since the yeast nuclear genome is A+T rich (about 39% G+C), the restriction enzyme *XhoI* (recognition sequence CTCGAG⁶) cuts yeast DNA relatively infrequently, generating fragments that are usually greater than 5 kilobase pairs (kb). An *XhoI* restriction digest of yeast DNA was transferred to nitrocellulose and hybridized to poly(dGdT·dCdA). Although many of the hybridizing bands were 5 kb or greater in size, a very prominent 1.4 kb band was observed. As shown in Fig. 1b, the intensity of this band indicated that it must be repeated in the genome. In addition, the band was always blurry relative to other bands in the digest, indicating heterogeneity within the 1.4 kb *XhoI* family (Figs 1b, 2a).

A repeated yeast sequence 1.4 kb in size was of particular interest since Szostak and Blackburn⁷ had shown that the yeast chromosomes have a conserved *XhoI* site 1.4 kb from the end. These workers had also demonstrated that the extrachromo-

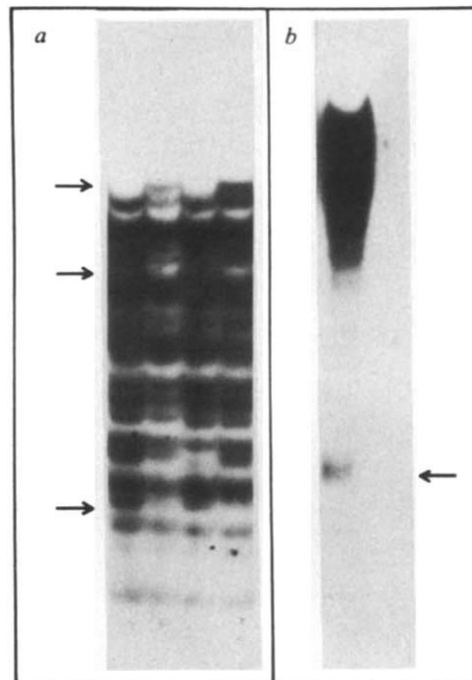


Fig. 1 Poly (GT) tracts in yeast DNA. *a*, DNA isolated from spore cultures derived from a single tetrad of the diploid strain +D4. Arrows indicate the position of fragments that differ in the spore cultures that show 2:2 segregation. *b*, DNA isolated from the haploid yeast strain A364a was treated with the enzyme *XhoI*. The arrow indicates the position of the 1.4 kb fragment derived from the telomere. **Methods:** The DNA was treated with the enzyme *MspI* and the resulting fragments were examined by Southern analysis. The filter with the DNA fragments was hybridized to poly(dGdT·dCdA) (Boehringer Mannheim) that had been labelled by nick translation with ³²P-dCTP. Hybridization was done at 55 °C for 16 h in Denhardt's solution with 10% dextran sulphate and without carrier DNA¹.

somal ribosomal DNA of *Tetrahymena* replicated in yeast as a linear DNA molecule. They isolated the yeast telomeric sequences by looking for yeast fragments that allowed replication of a linear plasmid with only one *Tetrahymena* end. Using the cloned yeast telomeric sequences as a hybridization probe, they showed that most telomeres contained an *XhoI* site 1.4 kb from the end of the chromosome although some evidence for heterogeneity in the size of this fragment was observed. Within this 1.4 kb end fragment, no restriction sites existed for the enzymes *PvuII*, *HindIII*, *ClaI* or *BamHI*. We found that the 1.4 kb fragment that hybridizes to poly(dGdT·dCdA) also was uncut by the same four enzymes (data not shown). The yeast telomeric sequences are cut by *PstI* and *KpnI* (B. K. Tye and C. Chan, personal communication). In double digest experiments, we found that these two enzymes also cut the 1.4 kb GT sequence (data not shown).

To prove that the yeast telomeres contain GT tracts, we performed two other experiments. First, we examined the ability of the 1.4 kb fragments to ligate into circular DNA molecules. Restriction fragments of yeast DNA internal to the chromosome ends at low DNA concentrations should be capable of ligation into circles. Restriction fragments containing the chromosome end should be incapable of circle formation but should be capable of ligation into multimeric linear forms. We treated yeast DNA with the restriction enzymes *XhoI* and *SalI*. Both *SalI* and *XhoI* leave the same 5' protruding end; the 1.4 kb *XhoI* fragment is uncut by *SalI*. Different concentrations of these DNA fragments (from 20 to 500 µg ml⁻¹) were treated with T4 DNA ligase and examined by Southern blot analysis. After hybridization to poly(dGdT·dCdA) (Fig. 2a), we found that the 1.4 kb band was missing from DNA samples that were ligated at high DNA concentrations, presumably because the 1.4 kb fragments ligated to other DNA molecules.

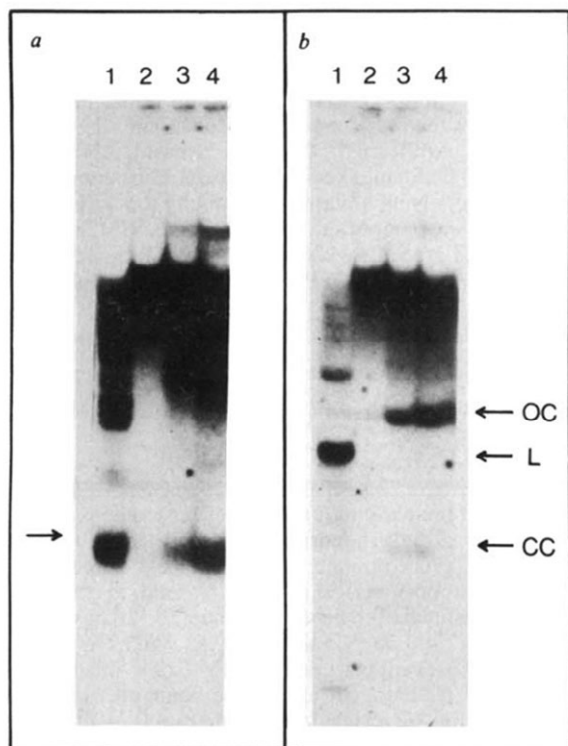


Fig. 2 Ligation of telomeric fragments. DNA was isolated from A364a and treated with the restriction enzymes *SalI* and *XhoI*. The DNA was divided into four 5- μ g aliquots and precipitated by ethanol. The samples were resuspended in a buffer containing 50 mM Tris-HCl (pH 7.8), 10 mM $MgCl_2$, 20 mM dithiothreitol and 1 mM ATP at concentrations of 500 μ g ml^{-1} (samples 1 and 2), 100 μ g ml^{-1} (sample 3) or 20 μ g ml^{-1} (sample 4). Eighty units of T4 DNA ligase (New England Biolabs) were added to samples 2, 3 and 4. After 16 h at 12 °C, the samples were concentrated into equal volumes, divided into two portions and examined by Southern analysis. One filter (a) was hybridized to ^{32}P -labelled poly(dGdT·dCdA); the second (b) was hybridized to a ^{32}P -labelled *SalI*-*EcoRI* restriction fragment that contained the yeast *leu2* gene¹². In a, the arrow indicates the position of the linear 1.4 kb *XhoI* telomeric fragment. The arrows in b indicate the expected position for the *SalI*-*XhoI* fragment containing the yeast *leu2* gene; the expected positions for the nicked circle, linear and covalently closed circle containing the *leu2* gene are indicated by OC, L and CC respectively. Additional bands in b are observed because the *SalI*-*EcoRI* fragment that was used as a hybridization probe was contaminated with a small amount of a second DNA fragment containing a repeated yeast gene *Ty1*.

In DNA ligated at low concentrations, the 1.4 kb band was clearly visible but no clear bands were visible below 1.4 kb. In the gel system used in these experiments, closed circular DNA molecules less than 5 kb in size migrate faster than linear DNA molecules⁸. Thus, failure to observe a fast migrating band suggests the 1.4 kb band is incapable of intramolecular ligation. As a control, the same DNA samples used in Fig. 2a were hybridized to a hybridization probe containing the yeast *leu2* gene (Fig. 2b). It is known that the yeast *leu2* gene is contained within a *SalI*-*XhoI* fragment of about 2.2 kb⁹. At low DNA concentrations, we found bands at the positions expected for both open circular and supercoiled DNA.

In a second experiment, we examined the ability of poly(dGdT·dCdA) to hybridize to the cloned yeast telomere. When uncut DNA was isolated from the strains A2 (wild-type yeast with no linear plasmid), T388 (a strain with a linear plasmid with two *Tetrahymena* ends) and T632.5 (a strain with a linear plasmid with one *Tetrahymena* end and one yeast end), we found hybridization to poly(dGdT·dCdA) at the position of the linear plasmids in T388 and T632.5 (Fig. 3a, b). This result shows that the *Tetrahymena* ends (when cloned in yeast) hybridize to poly(dGdT·dCdA). To demonstrate that the

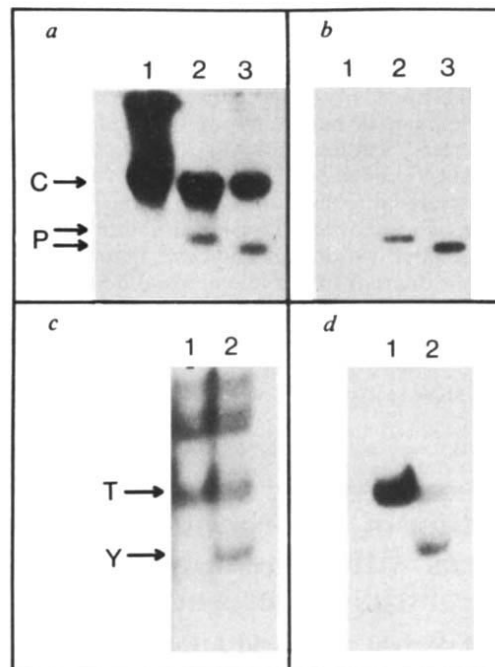


Fig. 3 Hybridization of poly(dGdT·dCdA) to the cloned yeast telomere. a, DNA was isolated from yeast strains A2 (wild type, no plasmid), T388 (linear plasmid pSZ216 containing two *Tetrahymena* telomeres) and T632.5 (linear plasmid pSZ219 containing one *Tetrahymena* telomere and one yeast telomere). Uncut DNA was examined by Southern analysis. Lanes 1, 2, 3 represent DNA isolated from A2, T388 and T632.5 respectively. The DNA on the filter was hybridized to ^{32}P -labelled poly(dGdT·dCdA). Position C represents chromosomal DNA and 'P' indicates the positions of the linear plasmids. b, The filter used in a was heated in 50 ml of 5 mM Tris-HCl at 80 °C to dissociate the labelled poly(dGdT·dCdA) and re-hybridized with ^{32}P -labelled pBR322. Plasmid pBR322 sequences are found in both pSZ216 and pSZ219⁷. c, DNA isolated from T388 (lane 1) and T632.5 (lane 2) was treated with the enzyme *PvuII* and examined by Southern analysis. The filter was hybridized to poly(dGdT·dCdA). *PvuII* cleaves pSZ216 from T388 into two fragments of 5.7 kb and 6.0 kb and cuts pSZ219 from T632.5 into a 5.7 kb fragment containing the *Tetrahymena* telomere and a 4.4 kb fragment containing the yeast telomere. Labels T and Y show the expected positions of the *PvuII*-cleaved *Tetrahymena* and yeast telomeres. d, The same DNA samples described in c were hybridized to ^{32}P -labelled pBR322.

cloned yeast telomere also hybridized to the probe, we treated the yeast DNA with *PvuII*. The linear plasmid in T632.5 is pSZ219⁷. The enzyme *PvuII* cuts this plasmid into two fragments, a 5.7 kb fragment containing the *Tetrahymena* end and a 4.4 kb fragment containing the yeast end⁷. As shown in Fig. 3c, d, both ends hybridize to poly(dGdT·dCdA). Control experiments showed that the observed hybridization was to the cloned ends rather than internal portions of the vector.

Thus poly(dGdT·dCdA) hybridizes to the telomeres of the yeast chromosome as well as to the ends of the *Tetrahymena* rDNA cloned in yeast. The simplest interpretation of this result is that the ends of these molecules contain (GT)_n tracts. Previously, Szostak and Blackburn⁷ showed that the yeast telomere has DNA strands that were rich in G and T residues and Blackburn and Gall¹⁰ observed that the *Tetrahymena* ends contained more than 20 C₄A₂ repeats. Our results indicate that the *Tetrahymena* ends contain a (GT)_n tract in addition to the C₄A₂ repeats. In order to exclude the possibility that the poly(dGdT·dCdA) probe was capable of hybridizing to the simple sequence C₄A₂, we did a Southern analysis of the plasmid pSZ221, which contains multiple C₄A₂ tracts but no (GT)_n tracts¹¹. We detected no hybridization to the homopolymer probe. We also found that extrachromosomal rDNA isolated from *Tetrahymena* (provided by E. Blackburn) did not hybridize to poly(GT). These results suggest that the probe is

likely to be specific for poly(GT) tracts in the genome and, in addition, that poly(GT) tracts are added to the ends of *Tetrahymena* rDNA when these molecules are cloned in yeast. It is likely that the tracts are added either by recombination with the yeast telomeres or by an enzyme that adds poly(GT) to a linear substrate. Szostak and Blackburn⁷ found evidence indicating that *Tetrahymena* ends cloned in yeast were larger than those isolated directly from *Tetrahymena*.

There are two important unresolved points relating to our observations. First, we do not know the nature of the (GT)_n tracts that are internal to the telomeres although preliminary evidence suggests that some of these tracts may be near but

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Integration of mitochondrial gene sequences within the nuclear genome during senescence in a fungus

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Cellular senescence in the ascomycete fungus *Podospora anserina* is associated with the appearance of an altered mitochondrial genome. Discrete mitochondrial DNA sequences are excised and amplified and isolated as multimerically arranged, head-to-tail repetitions^{1–4}. We have referred to the most frequently observed excision/amplification product as α -event senDNA^{5,6}. It is a 2.6-kilobase pair (kbp) monomeric unit (see refs 1, 3, 7) and is often found in senescent mitochondria in conjunction with other excision products. At the final stage of senescence these plasmids constitute virtually all of the DNA present in senescent mitochondria; they have replicated to high copy number at the expense of the young native genome. Because *P. anserina* is characterized by race-specific timing of senescence (that is, a programme of senescence), we have begun to contrast rapidly and slowly senescing races in terms of senDNA. Here we present evidence that young mitochondria of the rapidly senescing race, A⁺, possess an extremely high copy number of α -event senDNA plasmid in contrast to the more slowly senescing races s⁺ or s⁻. Moreover, we observe that during senescence the α -event senDNA and the β -event senDNA⁶ (a 9.8-kbp monomer) are transposed to the nucleus and integrated into nuclear DNA. These plasmids contain the coding information for subunits I and III (respectively) of the mitochondrial cytochrome c oxidase. This constitutes the first clear evidence for the active mobilization of genetic elements from the mitochondrion to the nucleus.

The presence in young, non-senescent s⁻ race mitochondria of autonomously replicating α -event senDNA has been reported previously⁷. Because of the obvious interest in examining senescence in terms of both the occurrence of senDNAs and the race-specific timing of senescence, we began to contrast the rapidly senescing (average 12 days) A⁺ race with the more slowly senescing (averages 40 and 25 days) s⁺ and s⁻ races. Again, as in the s⁻ race, we found frequent occurrence of the α -event senDNA in senescent A⁺ mitochondria. Previous reports^{7,8} suggested the absence of the plasmid form of α -event senDNA in the young s⁺ race mitochondria and in the s⁻ cap^R 1 mutant. We reexamined this issue with respect to both the s⁺ race and the A⁺ race. Some of the results are presented in Fig. 1 where the cloned α -event senDNA has been used as a nick-translated hybridization probe of Southern⁹-blotted mitochondrial DNA.

not at the chromosome ends (R.W., T.D.P., C. Chan, B. K. Tye, unpublished data). Second, the function of these tracts in relation to the telomere is obscure. Since poly(GT) can exist *in vitro* as Z form DNA², one interesting possibility is that Z-form DNA is required for telomere function.

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We observed the plasmid form of α -event sequences in both young A⁺ and s⁺ mitochondria. We also detected very high levels of the α -event plasmid in A⁺ senescent mitochondria. The relative proportions of α -event plasmid to the young genome were estimated to be approximately 1:20 in young s⁺, 1:1 in young A⁺ and 20:1 in senescent A⁺. We have observed the presence of α -event plasmid in young s⁺ or s⁻ in all cultures in which it has been sought and in the youngest cultures we can produce. Thus we conclude that either excision of α -event sequences occurs very soon after spore germination or the α -event plasmid is transmitted as a free plasmid via the spores. Furthermore, the high concentration of α -event plasmid in the A⁺ race, in contrast to the very low concentration of the plasmid in the s⁺ race, leads us to conclude that perhaps this concentration differential in young mitochondria constitutes a critical parameter for interpreting the senescence programme. Because of the complexity of the senescent phenotype we cannot yet propose a rigorous hypothesis in this regard, although the necessary work to do so is in progress.

We have previously characterized⁶ a new senescent event referred to as the β -event senDNA which has arisen several times independently as well as in conjunction with the α -event senDNA. The restriction map positions of the α -event and the β -event on the young mitochondrial genome are illustrated in Fig. 2. As shown previously⁶, these regions of the genome correspond to the coding sequences of the gene *oxi3* (subunit I of cytochrome c oxidase) and *oxi2* (subunit III of cytochrome c oxidase). The α - and β -event senDNA represents respectively the excision of the *oxi3* coding region and of the *oxi2* coding region and some flanking sequences. These will be described more fully elsewhere.

Both we^{4,6} (this report) and others^{7,10} have examined the young, non-senescent nuclear genome of *P. anserina* races s⁺, s⁻ and A⁺ for the presence of senescent plasmid sequences and have failed to locate such sequences. We have subsequently examined nuclear genomes from senescent mycelia of s⁺, s⁻ and A⁺ races for the presence of α - and β -event senDNAs. We report here the transposition of an amplified form of the α -event plasmid and of a monomeric form of the β -event plasmid to the nuclear genome during senescence. To demonstrate this, our strategy in α -DNA for example, was to digest senescent nuclear DNA with restriction endonucleases which have either a single site (*HaeIII*, *BglII* and *SalI*) or no site (*EcoRI* and *XhoI*). In this way, the integration of mitochondrial DNA would be revealed as plasmid DNA plus flanking sequences or as high molecular weight for *in situ* DNA. Some of these data are illustrated in Figs 3 and 4. In Fig. 3 we have used cloned, nick-translated α -event senDNA as the hybridization probe of restriction endonuclease-digested, Southern-blotted senescent nuclear DNA. We observe that the α -event probe hybridizes to nuclear DNA of very high molecular weight when the DNA is cleaved by an enzyme which does not cleave within the α -event sequences (*EcoRI* or *XhoI*) or when the nuclear

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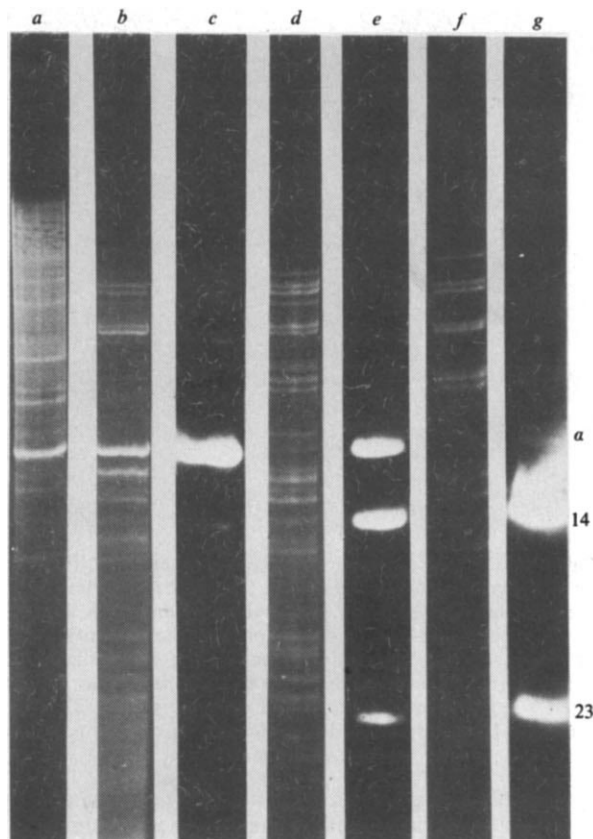


Fig. 1 Distribution of α -event senDNA. Mitochondrial DNA (mtDNA) was isolated and prepared from liquid cultures of *P. anserina* as previously described¹⁴. mtDNA was cleaved with the restriction endonuclease *Hae*III which cleaves the α -event plasmid at one site, and was electrophoresed on 1.2% agarose gels. The gels were stained with ethidium bromide and photographed (lanes a, b, d, f). They were blotted to nitrocellulose by the methods of Southern⁶, and the filters were hybridized to 5×10^5 c.p.m. of cloned, nick-translated¹⁵ α -event senDNA (lanes c, e, g). Filters were hybridized, washed, dried and autoradiographed as described previously⁵. Lanes a-c correspond to senescent A⁺ mtDNA; a and b are different isolates showing equivalent levels of α -event plasmid. Lanes d and e are A⁺ young mtDNA. Lanes f and g are s⁺ young mtDNA. Hybridization to the native, young genome is to *Hae*III fragments 14 and 23, previously indicated^{5,7} to be the excision site for the α -event, and these are marked on the figure. Hybridization to the linearized α -event plasmid is indicated. The interpretation that the α -event sequences indicated represent free plasmid rests on restriction analysis data, Southern blotting data, electron microscopy and cloning. These data have been reported elsewhere^{1-3,7}.

senDNA is not cleaved (data not shown). An enzyme which cleaves once within the α -event (*Bgl*II) produces three sites of hybridization: two faint flanking regions (f-1 and f-2) and one intense band at the position of the amplified, linearized plasmid. All enzymes tested which do not cleave within the α -event plasmid produce this high molecular weight signal and all enzymes tested which cleave within the plasmid release amplified, linearized plasmid and variably sized, low-intensity hybridization signals from the flanking DNA. We interpret this to indicate integration of the α -event senDNA into nuclear DNA in a highly repeated, head-to-tail form.

Among several interesting observations, there seems to be a higher copy number of α -event integration in the A⁺ race in contrast to s⁺ or s⁻ races, consistent with the situation in young A⁺ mitochondria. In addition, separate isolates of senescent nuclear DNA from s⁺ and s⁻ races exhibit identical hybridization patterns to linearized and flanking sequence DNA, suggesting a single site of integration for α -event senDNA in s⁺ or s⁻. It is evident from Fig. 3a, however, that not all senescent isolates necessarily exhibit nuclear integration of α -event

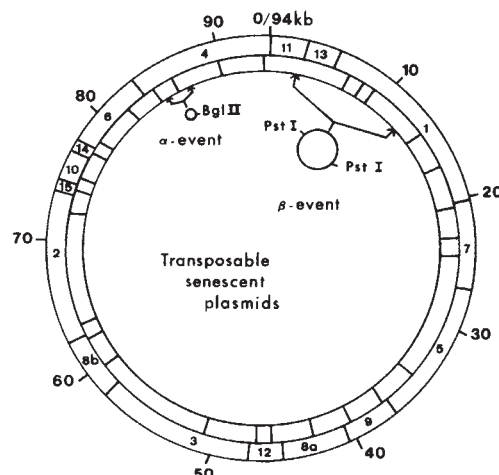


Fig. 2 Origin of α - and β -event senDNAs. The circular restriction maps for *Eco*RI and *Bgl*II are shown. *Eco*RI is outside; *Bgl*II is internal, and the numbering system for *Bgl*II is given in ref. 6. Excision events which gave rise to the α and β plasmid DNAs occurred by circularization of the young genome at the sites marked by arrows on the restriction maps. Thus, in cultures possessing both plasmid DNA and the young genome, a complete copy of the plasmid will be retained on the corresponding young genome and will show up as such in Southern blots such as that of Fig. 1. We emphasize that the designation of α -event and β -event refers specifically to plasmid DNAs derived from exact regions of the young genome and not to the buoyant density of the DNA⁷. Other discrete loci which also give rise to senDNAs (see refs 6, 8) are not shown here.

sequences. We also note that the flanking signals for the A⁺ race are often difficult to detect, and may reflect different integration from that in s. It is important to emphasize that the detection of α -DNA sequences is not simply the result of random degraded mitochondrial DNA because the excised α -DNA from the nucleus precisely co-migrates with the plasmid.

These results on the apparent integration of α -DNA mitochondrial gene sequences into the nuclear genome during senescence were so surprising that confirmatory evidence of a different sort was necessary. Belcour *et al.*^{8,11} have recently described a very interesting mitochondrial mutant in *P. anserina*, *mex1*. This mutant was isolated as an outgrowth of senescent mycelia. Restriction enzyme analysis showed that mitochondrial DNA from *mex1* completely lacked fragments *Hae*III 14 and 23, the exact region from which α -DNA sequences are derived. As it was possible that these cells possessed a functional *oxi3* gene integrated into the nuclear genome, we hybridized ³²P-labelled α -event senDNA to *mex1* mitochondrial and nuclear DNA. As reported^{8,11}, the mitochondrial DNA did not hybridize to the α -DNA plasmid probe. However, hybridization was readily detected in *mex1* nuclear DNA (Fig. 3, lanes i, j). Here we digested *mex1* nuclear DNA with either *Hae*III or *Sal*I endonucleases, enzymes which cleave α -plasmid only once but would be expected to cleave nuclear DNA to different extents. In each case, two fragments from nuclear DNA were detected. As expected, those from *Sal*I were of much higher molecular weight than those from *Hae*III. The presence of two fragments, neither of which co-migrated with α -plasmid, is strong evidence that these mitochondrial DNA sequences are integrated as single copy. It is not clear from these data why in senescent nuclear DNA, α -plasmid seems to be integrated in multiple copy whereas in *mex1* only single copy was detected, nor whether multiple sites of integration exist. Cloning these sites from races s and A as well as *mex1* nuclear DNA will be necessary to address both these questions. In other experiments we showed that *Hae*III-5, the fragment contiguous with *Hae*III-23^{5,6}, was completely contained in the *mex1* mitochondrial DNA, so it seems that α -event DNA is excised and transposed to the nuclear genome as a unique entity.

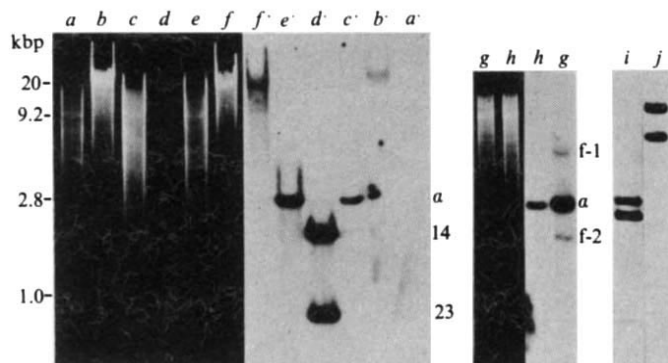


Fig. 3 Nuclear transposition of the α -event senDNA. Nuclear DNA (density 1.712 g cm^{-3}) was isolated from liquid cultures of *P. anserina* as described by Cummings *et al.*¹⁴ and was purified free of mtDNA (density 1.694 g cm^{-3}) by two cycles of CsCl-Dapi gradient centrifugation. Approximately $5 \mu\text{g}$ of DNA were cleaved with restriction endonucleases, electrophoresed on 1.2% agarose gels, stained with ethidium bromide, photographed and blotted to nitrocellulose by the methods of Southern⁹. Filters were hybridized to 5×10^5 c.p.m. of cloned, nick-translated α -event senDNA, washed, dried and autoradiographed as described previously⁵. Lane a, s^+ senescent nuclear DNA cleaved with *Bgl*II (cleaves once within the α -event); lane b, s^- senescent nuclear DNA cleaved with *Eco*RI (does not cleave α plasmid); lane c, s^- senescent nuclear DNA cleaved with *Bgl*II; lane d, s^+ young mtDNA cleaved with *Hae*III showing the origin of plasmid DNA on the young genome (s^+ and s^- have identical mtDNA, A^+ differs in *Hae*III fragments distinct from α -sequences (see Fig. 1); lane e, A^+ senescent nuclear DNA cleaved with *Bgl*II; lane f, A^+ senescent nuclear DNA cleaved with *Eco*RI; lane g, s^+ senescent nuclear DNA (a different isolate from lane a) cleaved with *Bgl*II; lane h, s^- senescent nuclear DNA (a different isolate from lanes b or c) cleaved with *Bgl*II. On the left of each set is the ethidium bromide pattern; on the right is the autoradiograph of the hybridization to α -event senDNA. Lanes i and j show the hybridization to *mex1* nuclear DNA digested with *Hae*III and *Sal*I restriction endonucleases, respectively. There is one site in α -event senDNA for each of these enzymes. Blot transfer of high molecular weight DNA was often less efficient than transfer of low molecular weight DNA, resulting in variably diminished signals in some lanes (b, f).

Figure 4 illustrates the β -event senDNA experiment analogous to that for α -event in Fig. 3. Again, the use of an enzyme to cleave the nuclear DNA which does not cleave within β -event senDNA (*Bam*HI) produces a high molecular weight hybridization signal, and an enzyme (*Bgl*II) which cleaves three times within β -event sequences releases two small *Bgl*II fragments and two large flanking sequences. The relative stoichiometry of internal and flanking sequence signals seems to be the same, and we interpret this to indicate integration of the β -event as a monomer. It is interesting that we obtain little detectable hybridization to the A^+ race but do detect good hybridization to the s^- race, the same isolate which showed α -event integration. Also, it does seem that the entire 9.8-kbp β -event senDNA has become integrated into senescent nuclear DNA. We are not certain whether integration of α -event precludes integration of β -event or vice versa, but the results with the s^- isolate used in Figs 3 and 4 suggest that this may not be the case.

Van den Boogaart *et al.*¹² have demonstrated the presence in the nuclear genome and in the mitochondrial genome from *Neurospora crassa* of very similar genes coding for the dicyclohexylcarbodiimide binding protein of mitochondrial ATPase. This observation differs markedly from our present report because in their situation the nuclear genes seems to be a stable component of the nuclear genome. As we report here for *oxi2* and *oxi3*, these genes are clearly absent from the young nuclear genome, but transpose to the nuclear genome during senescence. Note that we have also examined regions of the mitochondrial genome which have not arisen as senescent plasmids^{5,6}. As these regions (*Eco*RI-6 and -9) are not detected in



Fig. 4 Nuclear transposition of the β -event senDNA. This is the same experiment illustrated in Fig. 3 with the exception of the omission here of lanes g-j. The hybridization probe is cloned β -event senDNA. Lane d shows the origin of the β -event senDNA on the young mitochondrial genome. Lane c shows the nuclear homology of β -event senDNA; the flanking sequences are indicated (f-1, f-2) as are the two *Bgl*II fragments (see Fig. 2). In lane b nuclear DNA was cleaved with *Bam*HI which does not cleave within β -event senDNA⁶.

senescent nuclear genomes (data not shown), we feel that the occurrence of mitochondrial sequences as autonomously replicating plasmids is a prerequisite for nuclear transposition.

We have thus demonstrated that the race-specific timing of cellular senescence is correlated with the concentration of α -event senDNA in young mitochondria. More importantly, we have observed that during senescence the plasmids carrying sequences encoding mitochondrial *oxi2* and *oxi3* genes are transposed to and integrated into the nuclear genome. The connection between these two findings resides in the manner by which α -event senDNA may be related to senescence. High levels of mitochondrially located, self-replicating plasmid DNA could promote degeneration of mtDNA by homologous recombination and promote the degeneration of mitochondria with the subsequent release of senescent plasmids. The rate at which this process occurs could be a direct function of the concentration of plasmid sequences in the mitochondria, hence race-specific. Once free in the cytosol, senescent plasmids could readily move to the nucleus and undergo integration. This process may be analogous to the integration of the yeast 2μ plasmid DNA¹³ which promotes instability and degeneration of nuclear chromosomes, and perhaps this mechanism also operates in the mitochondria of *Podospira*. It will thus be interesting to determine the identity and sequence of the sites of nuclear integration and the potential consequences of this genetic mobilization during senescence in *P. anserina*.

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Safety is as safety is seen

Alvin M. Weinberg

The Cult of the Atom: The Secret Papers of the Atomic Energy Commission.

By Daniel Ford.

Simon & Schuster: 1982. Pp.270. \$13.95.

DANIEL Ford has written a powerful indictment of nuclear energy and its regulation, all the more devastating because it is so massively documented. Like a clever prosecuting attorney, he weaves together anecdotes drawn from hitherto unreleased papers of the Atomic Energy Commission to prove that the American nuclear establishment — with a few heroic exceptions — has been engaged in one big cover-up. According to Ford, the establishment has not ensured the health and safety of the public, nor has it been responsible in its regulation of what he regards as a profoundly flawed technology. Though he does not go so far as to urge that nuclear energy should be extirpated, *The Cult of the Atom* could, if read by enough uncritical readers, become nuclear energy's *J'accuse* or *Uncle Tom's Cabin*.

That nuclear energy poses serious risks as well as conferring important benefits was acknowledged in the Atomic Energy Acts of 1946 and 1954. At the time, some questioned the wisdom of vesting responsibility for both promotion and regulation of atomic energy in a single body, the Atomic Energy Commission. In retrospect, this concern was well-founded: and Ford bases much of his case on instances where the imperative to promote took precedence over the imperative to protect.

But the essential question is not, "Did the Atomic Energy Commission sometimes shade its decisions to favour promotion rather than protection?" — after all, where there is an inherent tension between "cheap as possible" and "safe as possible", decisions will always be close and disputable. It is rather, "Is the resulting technology safe enough?". And are there reasons to believe that the technology will become safer rather than less safe as more reactors are built? By omitting any balanced assessment of these crucial questions, Ford invalidates his case against nuclear power.

The two examples that together occupy prominent positions in his book — the Emergency Core Cooling System (ECCS) hearings of 1971–1972 and the Reactor Safety Study of 1975 — demonstrate the one-sidedness of Ford's argument. Whether the ECCS would douse the core of a reactor with water following a loss of coolant accident was, in the early 1970s, the central issue on which rested the assessment of the safety of Light Water Reactors. Ford is right in his claim that one AEC contractor placed pressure on researchers to shade their testimony at the

ECCS hearings so as to favour the industry position. But the 22,000 pages of the hearings transcript bear testimony that all relevant data were presented and thoroughly discussed. And Ford is dead wrong in implying that the safety of reactors was affected by the outcome of these hearings: the industry's position, rather than that of the critics, has turned out to be the closer to the truth. Two technical points were at issue: whether, following an accident due to loss of coolant, the fuel cladding would balloon out and thus block ingress of the emergency coolant; and second, whether the emergency coolant would bypass the active core entirely. That there was evidence to support both contentions (which would have been devastating to the nuclear business) is undeniable; but that the industry's position was also supported by analysis and experiment is also undeniable. The Commission, faced with conflicting evidence, chose to give most weight to the pessimistic evidence, and did not, as Ford suggests, generally support the claims of the nuclear industry.

All this happened ten years ago, and many experiments and analyses have been performed since then. Today we can say with confidence that the industry's position has been vindicated; the Commission decision — to reduce the heat rating during operation so as to limit the maximum temperature of the fuel cladding during a loss of coolant accident — has turned out, if anything, to be too conservative. The large-scale Loss of Flow Tests at Idaho Falls, in which the ECCS is tested in anger on a 50-megawatt operating reactor, has proved that the worries in 1971 about the adequacy of the ECCS were much exaggerated.

Ford's analysis of the controversy over probabilistic risk assessment — i.e. the *a priori* estimation by fault-tree analysis of reactor failure — can be similarly faulted, though perhaps not so unequivocally. The Commission and its successors could be criticized for suppressing the results of a 1967 study which showed that the worst core-melt accident could be a devastating catastrophe. Yet, the Commission's responsibility lay not merely in setting forth an estimate of the worst conceivable consequences, but also in giving some idea of how likely such an accident was. In the absence of such estimates of probability, I would insist that the Commission acted responsibly in *not* publicizing the estimated consequences. Not until 1975 did the

Commission have the final results of Professor Rasmussen's monumental Reactor Safety Study which, for the first time, gave estimates both of consequences and probabilities.

Was the Reactor Safety Study as unreliable and flimsy a document as Ford implies? Is the likelihood of an accident that causes very serious damage to the core around 5×10^{-5} per reactor year as claimed by Rasmussen on the basis of detailed study of two large reactors; or is it much higher, as claimed by Ford in his selective quotation of higher estimates; or is it totally indeterminate because Probabilistic Risk Assessment is simply too unreliable? Granted the large uncertainties, one can



Man at the core of the argument — Professor Norman Rasmussen of MIT.

still begin to compare experience with predictions. Three Mile Island-2 occurred after 400 reactor years of experience with Light Water Reactors (LWRs). This corresponds to a frequency fifty times higher than Rasmussen's median estimate. A retrospective analysis of the Reactor Safety Study by Rasmussen for a reactor of TMI-2 type led to an expectation value in agreement with this experience. Thus in this instance Rasmussen seems to have been vindicated, though he has revealed that some reactors are more prone to serious core damage than the two he studied originally. And about a dozen probabilistic risk assessments since TMI-2 of individual reactors yield a range of 10^3 in predicted core-melt probability between the best and the worst LWRs.

Curiously, Ford, as well as most other critics of nuclear power, finds much less fault with the estimates of the worst possible consequences of a reactor accident found in the Reactor Study than with the probabilities of such accidents, perhaps because the consequences are so alarming. Yet the consequences of even the worst accident — which must be far less probable than the probability of all core melts — appear to be greatly exaggerated, a point which Ford ignores entirely. The TMI-2 accident hurt no one. And many experiments (including the accident itself) point to the escape of a far smaller fraction of the iodine, tellurium and cesium fission

products than is assumed in the Reactor Safety Study. Moreover, the non-existence of a threshold for radiation-induced cancer, on which some of the most extreme calculations of consequences are based, is by no means as secure as critics of nuclear energy assume.

The future of nuclear energy depends on keeping the core-melt frequency low enough; and, as the number of reactors grows, the core-melt probability per reactor will have to fall correspondingly. In this respect Ford and I agree. But where I see the establishment of the Nuclear Safety Analysis Center and of the Institute for Nuclear Power Operations (which now encompass more than 90 per cent of all the world's reactors outside the Soviet bloc) as an effective means of enhancing knowledge and thereby reducing the likelihood of accident, Ford remains sceptical. And where I see the spectre of bankruptcy as a very strong incentive for reactor operators to correct deficiencies that have been revealed by Probabilistic Risk Assessments of specific reactors, Ford seems to dismiss this incentive. Yet when Probabilistic Risk Assessment revealed that the auxiliary feedwater system of the Calvert Cliffs reactor lacked enough back-up, the Baltimore Gas and Electric Company, largely on its own initiative, installed an additional auxiliary feedwater train.

One of Daniel Ford's themes is that good men were so blinded by visions of a golden age based on their technology, their transuranic elements, as to ignore the faults of their technology. In short, that overweening ambition and misguided enthusiasm have led to actions that, in his view, have been profoundly irresponsible. But Daniel Ford, as well as his colleagues in the anti-nuclear movement, bear an equally heavy weight of responsibility. To be sure, he is moderate in his actual recommendations — upgrading the safety of existing plants, curtailing the operation of some of them located in heavily populated areas, and generally stepping backwards. But in presenting an indictment that is so one-sided, he adds powerful impetus to the popular and political forces that have already halted nuclear energy in several Western countries. Is he, as well as *The New Yorker* magazine, which first published *The Cult of the Atom*, prepared to accept the full consequences of a rejection of nuclear energy?

The anti-nuclear community, as exemplified by Daniel Ford, has not discovered a practical alternative to nuclear energy. Unless such an alternative is found, I believe future generations will regard the nuclear establishment as having acted more responsibly than those who would find their fulfilment in the elimination of our only practical inexhaustible energy source. □

Alvin M. Weinberg is Director of the Institute for Energy Analysis of Oak Ridge Associated Universities, Tennessee. From 1955 to 1973 he was Director of Oak Ridge National Laboratory.

In short, innovation in technology

Bruce Williams

A Short History of Twentieth-Century Technology c. 1900–1950.

By Trevor I. Williams.

Oxford University Press: 1982. Pp.411. £12.50, \$25.

THE LAST two volumes of the seven-volume *History of Technology*, published by Oxford University Press in 1978, dealt with changes in technology from about 1900 to 1950. This short history by one of the editors of that work, Dr Trevor Williams, deals with the same period and is designed "to give the general reader a broad conspectus of the way in which technology developed and a glimpse of the social, economic and political factors that influence the development".

The book is well written, has excellent bibliographies at the end of each chapter, and includes many well-chosen illustrations. Explaining to the general reader the relevant science and engineering involved in, for example, nuclear weapons and power stations, antibiotics, selective weedkillers and insecticides, polymers, thermionic valves, transistors, computers and rockets is a formidable task which Dr Williams has managed most skilfully.

He is, however, less successful in providing a glimpse of the social, economic and political factors that influence the rates of change. In his introductory chapter he provides a sketch of the ways in which the spread of education in science and technology, the increases in Government support for R&D and in industry-financed industrial R&D, and changes in industrial organization contributed to higher rates of change. He also gives examples in several of his chapters of the ways in which — in the new environment — the interactions of "demand pull" and "technology push" stimulated change, but he could have provided a much more illuminating account had he paid closer attention to that distinction between invention and innovation which economists find so useful.

There are several references in the book to the influence of developments in technology on the way we live now, but — in striking contrast to the treatment of changes in technology itself — curiously few cross-references. For example, it is stated in Chapter 1 that "with the return of peace there seemed no limit to what science and technology could do for the benefit of mankind", though four chapters later there is a very good account of the development of the atom bomb, and in the penultimate chapter an account of military technology. The contrast between Longton in the Potteries in 1910 and 1970, shown by the illustration on the dust cover, is an excellent example of how the environment

can be influenced by changes in technology — in this case from coal-burning bottle kilns to electric and gas-fired ovens — and supporting legislation. Surprisingly, though, the only environmental issues dealt with in the chapter of public health are the improved technologies for storing and treating sewage, and for distributing water safe for drinking.

These limitations could be overcome in a second edition without a great increase in the size of the book. Nonetheless, if this first edition is judged on the basis of the author's intention — to provide a brief account of technological innovation and a glimpse of the social, economic and political factors that influenced it — that judgement should be very favourable. I certainly enjoyed the book and profited from reading it. □

Sir Bruce Williams is the Director of The Technical Change Centre, London, and Visiting Professor at Imperial College, University of London.

Immunoparasitology anew

F.E.G. Cox

Immunology of Parasitic Infections, 2nd Edn.

Edited by Sydney Cohen and Kenneth S. Warren.

Blackwell Scientific/C. V. Mosby: 1982. Pp.840. £48, \$99.50.

THE SIX years since the first edition of *Immunology of Parasitic Infections* was published have witnessed the emergence of immunoparasitology as a coherent discipline. It is no longer a subject which lags behind traditional immunology but now sets the pace itself.

The editors have faced the developments of those six years with courage and determination and the new edition is a completely different book from its forebear; few of the original chapters or authors appear in it. The section on immune responses to parasitic infections has been expanded from three chapters to six and coverage of immunodiagnosis has been reduced from ten chapters and incorporated into the relevant sections on specific infections, as has immunopathology which previously warranted four chapters.

The main section of the book now consists of fifteen chapters instead of ten. These deal with the more important parasitic diseases, this time including babesiosis and fascioliasis — which will make the book more useful for veterinarians and zoologists — and filariasis and giardiasis which were inadequately covered or omitted from the

earlier volume. The overall emphasis is largely medical and it is a pity that theileriosis, one of the most important cattle diseases in the world and one in which the immune responses have been superbly studied, is not dealt with.

The final section consists of two contributions on basic protozoology and helminthology. These are both competent and a vast improvement on the inadequate chapter in the first edition, but are not really necessary in a book of this kind.

Overall the new edition is much larger than its predecessor, both in page size and length, and it contains over twice as much material. It is attractively produced and

well referenced with most of the citations covering the period 1975–1981. This is a book that is a must for anybody working in immunoparasitology and also for other parasitologists and immunologists who want to find out what is happening in this area. It is also a book that must have been almost impossible to write and edit. Both the editors and the publisher are to be congratulated on producing a volume that is not only comprehensive but as up to date as modern publishing methods and the foibles of contributors can make it. □

F.E.G. Cox is Professor of Zoology at King's College, University of London.

57 varieties in the North American ark

Richard G. Van Gelder

Wild Mammals of North America: Biology, Management and Economics.
Edited by Joseph A. Chapman and George A. Feldhamer.
Johns Hopkins University Press: 1982. Pp.1,184. \$65, £40.

MANY wild animals are wanted as an exploitable resource, for hunting, to trap for fur, or for recreation, and people do not tolerate sharing their crops, trees, grazing lands, homes or livestock with them. Such animals are said to be of economic importance, and are subjects of the profession of wildlife management. That is why *Wild Mammals of North America*, a huge reference book and textbook, deals only with "57 of the economically important . . . species or species complexes . . ." of mammals in America north of Mexico.

The various authors of the sections follow a standard format for each of the kinds of mammals: nomenclature and distribution, description, physiology, genetics, reproduction, ecology, food habits, habitat, behaviour, mortality, age-determination, management, economic status and current needs for research and management, plus a bibliography. Maps of the present (as opposed to historical) distribution, drawings of skulls, photographs of animals and their parts or products, tables of measurements, graphs, lists of parasites, sex ratios and fur harvest data are among the extensive supporting material. The coverage is thorough and in parts very up to date — the average account is about 20 double-column pages long and a few references as late as 1981 are listed. The organization, type-faces and layout are clean and clear, with few typographical errors, and the book is easy to use. As to flaws, the photographs of skulls and animals are muddy, and the index follows the species-format too closely to be very useful.

Wildlife managers have come far from the knowledgeable, but untrained, outdoorsmen. Their outlook these days is broader and more professional, and they use more sophisticated devices for scientific study and monitoring animals than in the past. The profession's scope, too, is enlarging to include species not usually regarded as of "economic" importance, and many states now fund or include in their jurisdiction "non-game" species that were formerly the sole concern of academic or museum mammalogists. Because *Wild Mammals of North America* omits most of these ecologically important but "non-game" species (all 30 shrews, most bats, 128 species of rodents — among them prairie dogs, chipmunks, lemmings, kangaroo rats and pocket mice, and the ubiquitous white-footed mice — and some lagomorphs, cetaceans and carnivores), Chapman and Feldhamer's massive text may stand as the culmination of the "old", direct-impact approach of the profession. Of some 400 species of mammal in North America, only 190 receive even token coverage; given the editors' professed ecological "slant", some, if not all of this neglect, is questionable.

For reference and as a textbook, *Wild Mammals of North America* is excellent. As a guide to management philosophy, however, it is narrow. For example, when it is suggested on p.85 that a species of rabbit that has declined drastically in numbers and range should not be listed as endangered because its similarity in appearance to another important game species in the same area "could cause severe management problems" (i.e. curtail hunting), the credibility of wildlife managers as guardians of wildlife and ecosystems, as well as their scientific objectivity, becomes suspect. □

Richard G. Van Gelder is Curator of Mammals at the American Museum of Natural History, New York. His most recent book is Mammals of the National Parks (Johns Hopkins University Press, 1982).

Textbook supplement: next week's issue will include *Nature's* textbook supplement, containing reviews of some 120 books for students.

Beauty and the beast

Ian Stewart

The Fractal Geometry of Nature.
By Benoit B. Mandelbrot.
W.H. Freeman: 1982. Pp.440. \$32.50. £22.75.

MANY traditional models of Nature rest on the assumption that on a suitable scale of measurement there ceases to be any detailed structure. The success of calculus is in part responsible: a differentiable curve or surface is approximately linear, and a line or plane lacks structural features. But geometric forms can possess structure on all scales and at the turn of the century mathematicians constructed them in great numbers. They called them "pathological" and interpreted them accordingly as undesirable monsters serving merely to show the nastiness of Mathematics Untamed.

What is now becoming clear is that monsters too have their place in mathematical modelling; indeed that they are not monsters at all but undeservedly neglected creatures of great value and beauty. The awareness of this has largely come about by the efforts of one man, Benoit Mandelbrot, the author of this book.

In the days when monsters *were* monsters, their main implication was the
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inadequacy of the idea of "dimension" as "number of parameters required". They led to a wider concept, the Hausdorff-Besicovitch dimension, capable of taking non-integer values. Mandelbrot coined the apt term "fractal" to denote any set whose Hausdorff-Besicovitch dimension differs from its topological dimension. He also perceived the potential applicability of fractals to the geometry of Nature. Coastlines, landscapes, the bark of trees, cell tissue, the craters on the Moon: all are arguably better modelled by a fractal than by anything smooth. Striking evidence for this view may be seen in some of the colour plates in the book — artificial craters, mountains and fjords — all utterly lifelike and convincing.

But fractal geometry runs deeper. It commonly occurs in any process having

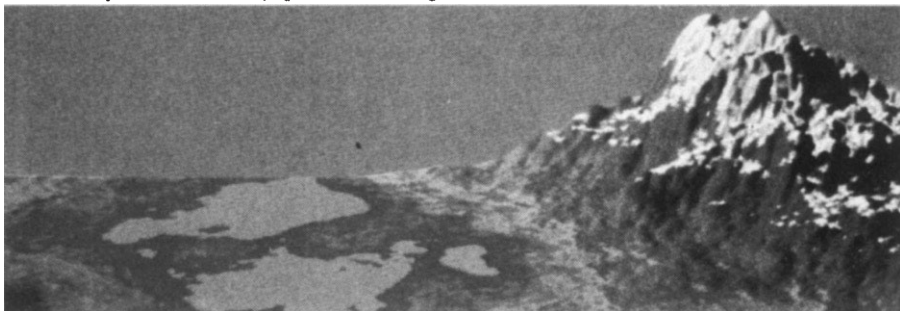
self-similarity, that is a structure (either exact or statistical) invariant under suitable scale changes. A coastline, magnified, still looks like a coastline but so do error-bursts in message transmission, energy cascades in turbulence and Brownian motion. Some techniques in physics tackle structure on many scales in a way not unsympathetic to a fractal view of the world, the renormalization group being a good example.

It is impossible to categorize this book. It is a blend of erudition (fascinating and sometimes obscure historical minutiae abound), popularization (mathematical rigour is relegated to appendices) and exposition (the reader need have little knowledge of the fields involved). Its author rightly calls it a "casebook" and a "manifesto", documenting the uses made of fractals to date and urging their

employment elsewhere. It is in addition a work of great visual beauty and the illustrations include many superb examples of computer graphics that are works of art in their own right. Mathematicians often say that mathematics is beautiful but here the beauty is evident to even the most casual reader. For this reason alone the book deserves very wide circulation indeed; but it can be recommended for another, more important, reason in that it presents a new point of view.

The theory of fractals is not yet fully mature. Many of its applications to date are purely descriptive, and some may question the relevance of fractal theory in the absence of any truly dramatic evidence of its practical importance. A notable lack is that of a theoretical underpinning, of reasons *why* fractals appear in certain processes, especially fluid turbulence. Possibly the theory of chaotic dynamics and strange attractors (which the author sensibly suggests be renamed fractal attractors) can be brought to bear. But none of this should detract from imaginative pioneering work. The great achievement of Mandelbrot is to have sensitized mathematicians and scientists to the fractal viewpoint, and to have pointed out the existence of a whole new and important regime for mathematical modelling. □

Ian Stewart is a Lecturer in Mathematics at the Mathematics Institute, University of Warwick. He is author of Les Fractals, a cartoon book published in French by Belin.



From *The Fractal Geometry of Nature*.

"Non-Gaussian hills that never were" — a computer generated landscape based on a version of Brownian motion related to the Gaussian distribution. The surface is a fractal of dimension greater than 2. The zero level, chosen arbitrarily, has been interpreted as the surface of a lake. "Snow" is added to the higher elevations by a secondary colouring routine. In the original, colour illustration, the lake is blue, passing through green on the shores, shades of brown, to white on the mountain's peak.

Darkness in Asia

Kenneth Mellanby

The State of India's Environment 1982: A Citizens' Report.

By Anil Agarwal, Ravi Chopra and Kalpana Sharma.

Centre for Science and Environment/ Ravi Chopra, New Delhi: 1982. Pp. 172. Price not available.

"INDIA is rapidly becoming a vast wasteland. Indians cannot now close their eyes to the continuing degradation of their natural environment". This quotation from its summary states bluntly the conclusions of this depressing book. It is an unofficial report, sponsored by various voluntary bodies, and has been edited and largely written by three Indian authors — Anil Agarwal, Ravi Chopra and Kalpana Sharma — with the help of some 30 other Indian scientists who are concerned with their country's environment.

These conclusions will make many readers despair, and adopt the views of some environmental writers of the late 1960s. Then it was suggested that India could never provide its people with decent living conditions and thus should be written off in global plans for development

and recovery. While some optimism has been engendered by improvements in many Western countries, it appears that the situation in India continues to get worse.

Although some progress in science, and some industrial and agricultural improvement is reported, this is said to be at the cost of environmental damage. Thus vast amounts of soil are washed away by the monsoons, reducing agricultural potential. Water pollution gets worse — 70 per cent of Indians drink filthy water and fish are poisoned while the government does little to enforce controls. Over a million hectares of forest are cut each year; planting makes up for only a fraction of this loss. Many ambitious schemes to dam rivers have had disastrous results. The air in the cities gets more polluted, levels of sulphur dioxide rising above internationally accepted standards. Disease control makes little progress — old diseases such as malaria are still rampant, new ones, including some forms of cancer, are increasing — and the death rate is no longer falling. Firewood still provides cooking energy for 90 per cent of the population but is becoming scarce. The unique wildlife is threatened.

Western readers will be surprised to find almost no comment (except for the statement that urban numbers have doubled in the last 20 years) on the subject of

population. No reference is made to the efforts of India's government, not entirely unsuccessful, to reduce the rate of increase. The chapter on "People" where such information might be expected deals with the plight of fisherfolk and nomadic tribes. The authors apparently do not agree that most of the damage to the environment is connected with the vast increase in the number of Indians.

The solution proposed by Agarwal, Chopra and Sharma is to build up self-reliance at the community level, and to develop a civilization following the ascetic ideals of Mahatma Gandhi. Whether this is practicable, and whether the citizens of the world's second largest nation can be persuaded to eschew the ideals of the consumer society remains to be seen. Nonetheless the fact that these Indian writers can make this proposal is important — it would not have been acceptable from expatriate "experts". At the same time there is a risk that some of these idealistic proposals will encourage old customs and an even higher birth rate. This could, in my opinion, have ramifications which would make the environmental degradation here reported look almost insignificant. □

Kenneth Mellanby is an Honorary Professorial Fellow at University College, Cardiff, and editor of the journal Environmental Pollution.